THERAPEUTIC POTENTIAL OF *Citrullus lanatus* RIND EXTRACT ON CADMIUM-INDUCED HEMATOLOGICAL ALTERATIONS IN WISTAR RATS

BY

Ifeoma Blessing MGBAKOGU (Miss)

LSC2007315

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

(PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES)

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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CERTIFICATION

This is to certify that this project titled "**THERAPEUTIC POTENTIAL OF Citrullus lanatus RIND EXTRACT ON CADMIUM-INDUCED HEMATOLOGICAL ALTERATIONS IN WISTAR RATS"** was carried out by **Ifeoma Blessing MGBAKOGU** with matriculation number **LSC2007315**, of the Department of Science Laboratory Technology (Physiology/Pharmacology), Faculty of Life Sciences, University, Benin City, Edo state, Under the supervision of DR. O. C. EKHATOR.

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DR. O. C. EKHATOR
PROJECT SUPERVISOR Date

____________________________ ____________________

DR. P.O. ALONGE
PROJECT COORDINATOR Date

____________________________ ____________________

PROF. J. O. OSARUMWENSE
HEAD OF DEPARTMENT Date

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EXTERNAL EXAMINER Date

DEDICATION

This work is dedicated to God Almighty

ACKNOWLEDGEMENT

To God almighty, the giver and sustainer of life, the owner of the universe, he who has never left my side throughout this journey thank you for giving me the grace to complete this work.

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ABSTRACT

This study investigated the therapeutic potential of hydroethanolic extract of watermelon (*Citrullus lanatus*) rind against cadmium-induced hematological alterations in Wistar rats. Twenty five rats were divided into five groups: a control group, a negative control group administered cadmium chloride (15mg/kg), a positive control group treated with Vitamin C (5 mg/kg), and two test groups treated with the watermelon rind extract at doses of 250 mg/kg and 500 mg/kg, respectively. The experiment lasted for 60 days. Results showed that cadmium exposure resulted in the lowest percentage body weight gain (37.41%), while treatment with the 500 mg/kg extract promoted the highest weight gain (84.66%), comparable to the Vitamin C group. Although blood cadmium levels did not show a statistically significant reduction, hematological analysis revealed that the extract, particularly at 250mg/kg, appeared to ameliorate cadmium-induced thrombocytopenia by restoring platelet counts towards normal levels. Other hematological parameters, including red and white blood cell indices, showed no significant alterations. The result suggest that *Citrullus lanatus* rind extract possesses protective properties against cadmium-induced toxicity, potentially through its rich antioxidant phytochemicals, which mitigate oxidative stress and support hemopoietic function, rather than through direct chelation of the heavy metal.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Heavy metals (HMs), like Lead and Cadmium pose a serious hazard to the environment and are a major global concern (Zhu *et al*., 2020). Heavy metal pollution of the atmosphere is a hazard for human health and is a result of rapid industry and urbanization (Nour *et al*., 2019; Liu *et al*., 2020). Because of their non-biodegradability, bioaccumulation, environmental stability, persistence, and biotoxicity properties, heavy metals (HMs) represent a significant environmental risk to living things and their environments (Khan *et al*., 2019; Zhang *et al*., 2019). They can directly affect the physical and chemical characteristics of sediment, soils, and water to stop microbial activity [8]. Through the food chain, they can also have a severe and long-lasting effect on the human body and disturb the natural ecology (Lian *et al*., 2019). Through the food chain's amplification effect, the non-biodegradable heavy metals (HMs) can also build up in the surface sediment for an extended period of time, leading to a number of illnesses and difficulties in humans (Zhu *et al*., 2020).

The spread of Cadmium to the marine habitats of aquatic ecosystems like rivers and estuaries is largely caused by both natural (such as geological weathering, atmospheric precipitation, wave erosion, wind, and bioturbation) and man-made (such as rapid industrialization, urbanization, agricultural runoff, and transportation) factors (Nour *et al*., 2019). Furthermore, HMs accumulate and sink in aquatic habitat surface sediments as a result of human activities that might cause industrial pollution, the deposition of urban garbage, and the offensive use of chemical fertilizers and pesticides (Kinimo *et al*., 2018). Water quality (Ali *et al*., 2020), and surface sediments that change environmental factors like pH, temperature, and bioturbation are negatively impacted by the HMs discharged into the water column. As a result, sediment quality can be crucial in determining the impacts of both natural and man-made activities (Nour *et al*., 2017). It can also inform environmental policy and management and reveal information about the impact of man-made activities on the ecosystem. Farm waste, agricultural runoff, pesticides, solid waste, waste management, effluents from fish processing plants, jute processing, cement manufacturing, oil refining, fertilizer manufacturing, building materials, soup and detergent factories and brickyard waste are the major sources of pollution. Due to the potential risk of HMs in water, soil and sediment through the disposal of the effluents mentioned, this riverine water, sediment and environment may be important (Tian *et al*., 2017).

Because of their wide variety of bioactive components, medicinal plants, often known as herbs, are essential for advancing human health and sustainable healthcare practices. These botanical resources, which have their roots in tradition and wisdom, provide a holistic and nature-based method of enhancing human existence. The importance of medicinal plants in promoting wellbeing is attributed to the wide variety of bioactive chemicals they contain (Gurib-Fakim, 2006). Terpenoids, polyphenols, alkaloids, and flavonoids are examples of compounds with medicinal properties. Their great importance stems from their diversity as well as their many functions, which range from anti-inflammatory drugs to antioxidant defenders protecting cells from oxidative damage to calming the body's uncontrollable reactions (Guo *et al*., 2024). In addition to their antioxidant properties, many medicinal plants also possess anticarcinogenic properties, meaning they can inhibit the growth and spread of cancer cells (Aye *et al*., 2019). Watermelon (*Citrullus lanatus*), a widely consumed fruit in many parts of the world, is known for its high water content and nutritional value (Benmeziane and Derradji, 2023). Interestingly, the peel of watermelon, often discarded as waste, contains various bioactive compounds such as flavonoids, phenolics, and alkaloids that exhibit antioxidant and therapeutic potentials (Nadeem *et al*., 2022). These compounds may help in scavenging free radicals, reducing oxidative stress, and restoring organ function compromised by heavy metal toxicity (Benmeziane and Derradji, 2023)

1.2 STATEMENT OF PROBLEM

Cadmium, a toxic heavy metal, poses a significant threat to human and environmental health due to its persistence, bioaccumulation, and potential to induce severe physiological damage. Exposure to cadmium, often through contaminated water, food, or industrial pollution, can lead to organ toxicity, oxidative stress, and various chronic diseases. Conventional treatments for heavy metal toxicity are often limited by side effects and cost, requiring the need for safer, more accessible therapeutic alternatives.

1.3 JUSTIFICATION OF THE STUDY

This research shows the need to develop natural, affordable, and effective countermeasures against heavy metal toxicity. Medicinal plants and their extracts are rich in bioactive compounds with antioxidant, anti-inflammatory, and detoxifying properties, showing a promising way for therapeutic intervention. Watermelon (*Citrullus lanatus*) rind, typically considered waste, contains beneficial phytochemicals that may help combat oxidative stress and organ damage caused by cadmium exposure. By investigating the therapeutic potential of this extract, the study not only contributes to environmental and biomedical science but also promotes the sustainable use of agricultural by-products, potentially adding value to food waste while addressing a significant public health concern.

1.4 AIM

To evaluate the therapeutic effect of Hydroethanolic extract of *Citrullus lanatus* on cadmium-induced hematological alterations in Wistar rats

1.5 The specific objectives are to:

1. evaluate the hematological parameters of the rats; and
2. evaluate the heavy metal level in the blood

CHAPTER TWO

LITERATURE REVIEW

2.1 CADMIUM

Along with arsenic, lead, mercury, and chromium, cadmium (Cd) is a heavy metal that has no physiological purpose and is frequently regarded as poisonous (Sinicropi *et al*., 2010; Sinicropi *et al*., 2020). Over the past century, numerous exposures to cadmium have been documented, and cadmium is found in the environment due to a variety of human activities (Rahimzadeh *et al*., 2017). Cadmium's use as a corrosive reagent in industry, as well as as a stabilizer in PVC products, color pigments, and Ni-Cd batteries, are the consistent sources of cadmium pollution (Genchi *et al*., 2020). House dust is a possible source of cadmium exposure in regions with polluted soils (Hogervost *et al*., 2007). Anthropogenic sources of Cd in the environment include the smelting and refining of copper and nickel, the burning of fossil fuels, and the use of phosphate fertilizers; cadmium is also found as a pollutant in non-ferrous metal smelters and in the recycling of electronic waste; rising concentrations of Cd in the living environment (atmosphere, soil, and water) are caused by volcanic activity, the slow erosion and abrasion of rocks and soil, and forest fires; even zinc, lead, and copper mines contribute to the atmospheric release of this metal, contaminating soil (Casado *et al*., 2008). Cd is mostly absorbed through the respiratory system and, to a lesser amount, by the gastrointestinal system; skin absorption is comparatively uncommon. After entering the body, cadmium is carried by erythrocytes and albumin into the bloodstream, where it builds up in the kidneys (Satarug, 2018), liver, and stomach. The body slowly excretes cadmium through the kidneys, urine, saliva, and milk during lactation. Numerous negative consequences, including renal and hepatic dysfunction, pulmonary edema, testicular injury, osteomalacia, and harm to the adrenal glands and hemopoietic system, can arise from exposure to Cd in humans (Tinkov *et al*., 2018).

Cadmium exposure markers (blood and urine) were also linked to coronary heart disease, stroke, peripheral artery disease, and atherogenic changes in lipid profile. Cadmium is a known human carcinogen (group I of the International Agency for Research on Cancer classification), and exposure to it at work or in the environment has been linked to cancers of the lung, breast, prostate, pancreas, urinary bladder, and nasopharynx (Mezynska and Brzoska, 2018). Cadmium-induced apoptosis is caused by the production of reactive oxygen species, buildup of Ca2+, downregulation of bcl-2, and p-53 deficiency.

2.2 CHEMICAL PROPERTIES OF CADMIUM

In the periodic table of elements, cadmium (Cd; atomic number 48, atomic weight 112.41) is a member of group XII. The physical and chemical characteristics of this soft, silvery-white metal are comparable to those of zinc and mercury. The combination of eight stable isotopes gives cadmium its atomic weight. It is a post-transition metal with a complete d orbital and two electrons in the s orbital. Furthermore, in the case of zinc, the oxidation state of +2 is preferred by the ductile, soft, and malleable cadmium in the majority of its compounds. In addition to being water-insoluble and non-flammable, cadmium is utilized as a protective plate and is resistant to corrosion. Cadmium oxide is created when cadmium burns in air. Due to its widespread occurrence, cadmium is found in measurable amounts in food, drink, and breath (Sinicropi *et al*., 2010). Cadmium is found in nature at low concentrations, primarily with the sulfide ores of zinc, lead, and copper. Cadmium is used as a pigment, stabilizer of PVC and alloys, and in the electroplating process that protects steel from corrosion. The environment is contaminated by cadmium due to human activities like burning fossil fuels, leachate from landfills, agricultural land, and mining waste, particularly from zinc and lead mines (Sinicropi *et al*., 2010).

2.3 EXPOSURE TO CADMIUM

The fast growth of industry and contemporary technologies has resulted in the introduction of cadmium as a contaminant into the environment (Satarug, 2019). High levels of it are absorbed from contaminated food, drink, and air. Crustaceans, bivalve mollusks, oysters, cephalopods, and crabs all have high levels of Cd, as do offal products including liver and kidney, oil seeds, cocoa beans, and some wild mushrooms (Satarug, 2018). Plant-based foods typically have higher levels of Cd than meat, eggs, milk, and dairy products, depending on the degree of soil contamination. Compared to other plant-based foods, the metal can be found in higher amounts in rice, wheat, green leafy vegetables, potatoes, carrots, and celery. Among these, rice and wheat, green leafy vegetables, potatoes, carrot, and celery can contain higher concentrations of the metal than other food from plants. Vegetarians and shellfish consumers may be exposed to a higher cadmium intake than omnivores. One of the major routes for cadmium exposure in humans is through rice consumption (Horiguchi, 2019). Because of this, flooding rice fields before harvest is advised as a water management technique to decrease the accumulation of Cd in rice, particularly in Japan, in regions where the soil is contaminated with Cd. However, this approach may increase the accumulation of As in rice (Arao, 2019). Because of its unique hydrochemical properties, cadmium may be mobile in groundwater. Unlike other heavy metals, which usually fix at pH values close to neutral (<6.5), it stays in solution (Kubier *et al*., 2019). As a result, Cd contamination of drinking water and rivers can happen, particularly in areas close to mines or factories. Tobacco use is another environmental factor that exposes the general public to Cd (Kubier *et al*., 2019).

Depending on the type of cigarette, smoking might expose one to different levels of cadmium. A person who smokes 20 cigarettes a day will absorb roughly 1 µg of Cd, as each cigarette contains 1-2 µg of the metal. With a 40%–50% lung absorption rate, about 10% of Cd is inhaled. Lung cancer and other smoking-related respiratory conditions are associated with inhaled cadmium. Mona *et al*. discovered a substantial increase in Cd in the serum and urine of smokers compared to non-exposed smokers (Mona *et al*., 2018). They discovered a considerable increase in bone pain percentage in smokers compared to non-exposed smokers (95% vs. 37.7%) and they concluded that long-term exposure to Cd may have an osteotoxic impact, resulting in bone tissue loss. Deterioration of vision and hearing, as well as harm to the kidneys, liver, skeletal system, and cardiovascular system, can result from low levels of cadmium exposure. Cadmium exhibits negative effects at low concentrations on human male and female reproductive, as well as affecting pregnancy or its outcome, in addition to its potent teratogenic and mutagenic effects (Kumar and Sharma, 2019). This is because numerous genes in the embryo have altered expression, which causes abnormal methylation of those genes in both the embryo and the placenta. Cadmium's capacity to readily bind to thiols with the depletion of the methyl donor S-adenosyl methionine has been connected to the epigenetic modification patterns it induces. This leads to changes in the methylome and, ultimately, in the activity of DNA methyltransferase.

2.4 CADMIUM AND PLANT'S PHYTOCHELATING CAPACITY

Naturally occurring biologically active substances found in plants may help avoid the harmful health impacts of exposure to heavy metals, such as cadmium. Polyphenolic compounds, melatonin, carotenoids, quercetine, resveratrol, vitamin E, vitamin C, L-carnitine, and coenzyme Q10 are some of these exposure chemicals (Wang *et al*., 2013; Ibrahim *et al*., 2014). The polyphenols and antioxidants found in fruits, vegetables, and spices, as well as in beverages like red wine, green tea, and chocolate, are among these compounds that show the most promise (Wang *et al*., 2012; Al-Gnami, 2014). Polyphenols' potent antioxidant qualities and capacity to chelate cadmium ions are the sources of their protective impact. Polyphenols reduce the absorption of this xenobiotic metal from the gastrointestinal tract because of the strong affinity of cadmium ions for hydroxyl groups and their capacity to upregulate intestinal MT expression (Brzóska *et al*., 2016). Antioxidants must be utilized with caution, though, if Cd toxicity is present. Melatonin alone and melatonin combined with vitamin E and selenium both exhibited protective benefits against oxidative damage caused by cadmium in rat liver, as shown by Kara *et al*. (2008).

2.5 CADMIUM'S EFFECTS ON MITOCHONDRIA AND Cd^{2+}-INDUCED APOPTOSIS

By producing adenosine triphosphate (ATP), the energy required for life, through oxidative phosphorylation (OXPHOS), the mitochondrial respiratory chain plays a crucial part in preserving energy balance. Furthermore, ion homeostasis, motility, apoptosis (programmed death), lipid and phospholipid synthesis, and amino acid synthesis are all related to mitochondria. Animal tissues and cells have different numbers and types of mitochondria depending on their energy requirements and how they react to changes in the environment or body. Aging and a number of illnesses, including cancer, are linked to malfunctioning mitochondria (Modica-Napolitano *et al*., 2007). The outer and inner membranes, the intermembrane gap, and the matrix are the four subcompartments that make up mitochondria. Post-translational changes including phosphorylation, ubiquitination, and sumoylation also affect the mechanism of mitochondrial morphology. Their genome, which is made up of a tiny double-stranded circular DNA molecule (mtDNA), is found in mitochondria. Thirteen of the 37 genes (16,569 base pairs) on the human mitochondrial chromosome encode respiratory chain proteins; the other twenty-four genes encode rRNA and tRNA, which are vital for the mitochondria's protein-synthesising apparatus. Other mitochondrial proteins (enzymes and membrane transporters) are encoded by nuclear genes. Five to seven genomes are typically found in mitochondria, and these genomes are stabilized by DNA-binding proteins distinct from nuclear histones. The thiol groups (-SH) of cysteines found in proteins are the essential targets of cadmium. Enzyme sulfhydryl group inactivation can result in a number of functional deficiencies in mitochondria, the endoplasmic reticulum, and nuclei. Because complex III (cytochrome bc1 complex or ubiquinone: cytochrome c oxidoreductase) hinders electron transport, the blockage of the mitochondrial electron-transfer chain is the primary cause of the toxic impact associated with cadmium. Cadmium causes a mitochondrial permeability transition pore to open, which increases ion permeability in the inner mitochondrial membrane and decreases respiration triggered by ADP and uncoupler (Belyaeva *et al*., 2008).

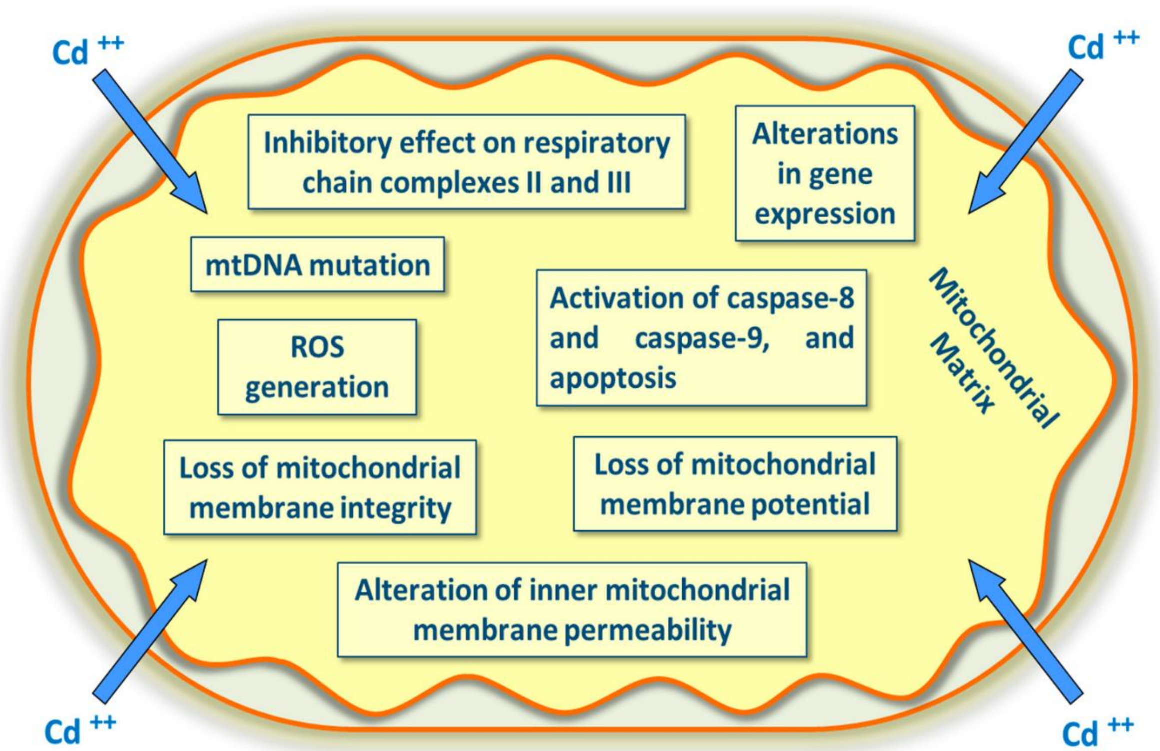

Plate 2.1: Cadmium intoxication (Chatterjee *et al*., 2008)

2.6 CADMIUM TOXICITY AND OXIDATIVE STRESS

The amount of cadmium in living things rises with age and food, and it has a significant cumulative effect in people (Satarug *et al*., 2017). The length of uptake, the metal's chemical form, the food, the age, sex, and health status of those exposed are some of the variables that affect the absorption and buildup of this xenobiotic (Nishijo *et al*., 2004). This heavy metal disrupts the homeostasis of vital biometals, including calcium, iron, magnesium, zinc, and selenium, as well as their biological processes (Wang *et al*., 2013; El-Boshy *et al*., 2015). The amount of these vital components, as well as vitamins, polyphenols, antioxidants, and other active biomolecules, in the diet determines how well this metal is absorbed by the digestive system. While a lack of some of these physiologically active substances can increase gastrointestinal absorption and the buildup of Cd in the body, an increased intake of certain bioelements may prevent Cd absorption and its toxic effects (Brzóska *et al*., 2016). One of the most hazardous heavy metals is cadmium, whose toxicity can be categorized as multidirectional. Cadmium ions show a significant attraction for biological molecules containing –SH groups (cysteine and glutathione GSH), as well as disulfide –S–S– groups (cystine and reduced glutathione GS-SG), causing impairment of their activities. The bivalent cadmium cation is unable to form free radicals directly; however, after cadmium exposure, there is enhanced creation of ROS, especially superoxide radicals, hydrogen peroxide, and hydroxyl radicals. Oxidative stress is induced by cadmium (Matović *et al*., 2015).

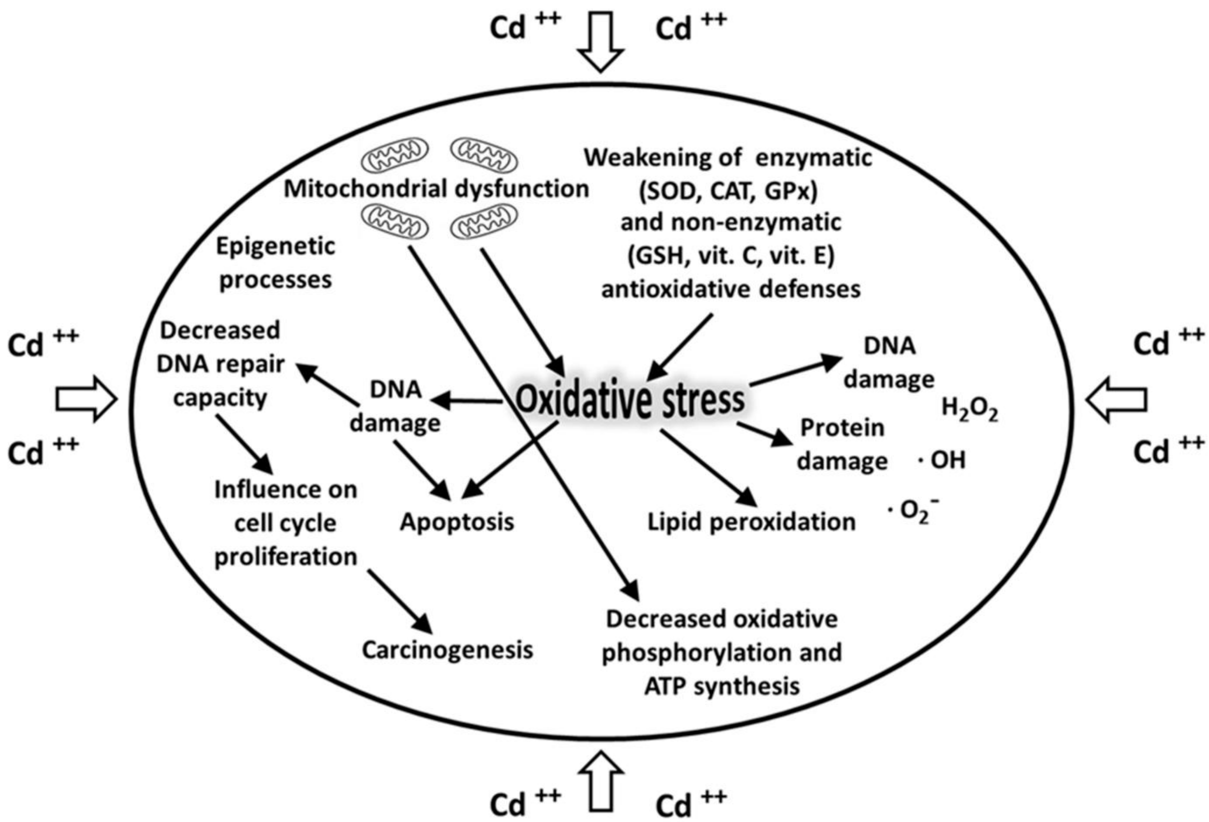

Plate 2.2: Oxidative stress and Cadmium toxicity (Chatterjee *et al*., 2008)

2.7 EFFECT OF CADMIUM ON THE HEMATOLOGICAL PARAMETERS

Cadmium (Cd) exposure demonstrates significant, albeit complex, effects on various hematological parameters and immune cell functions. *In vitro* studies on human peripheral blood leukocytes (PBLs) reveal that at environmentally relevant concentrations (3.1–16 µg/L), cadmium does not significantly affect cell viability or monocyte phagocytic activity (Fortier *et al*., 2008). However, its impact on lymphocyte proliferation is ambiguous; while some studies report inhibition of proliferation in response to mitogens like phytohemagglutinin (PHA) at concentrations approximating those in occupationally exposed subjects (Borella *et al*., 1990), the data from Fortier *et al*. (2008) indicated that non-cytotoxic concentrations of Cd, individually, did not modify the ability of lymphocytes to proliferate in response to anti-CD3 antibody. Similarly, natural killer (NK) cell cytotoxic activity was found to be inhibited by Cd in other studies (Cifone *et al*., 1990), although Fortier *et al*. (2008) noted no significant effects on NK activity from Cd or lead (Pb) individually, in contrast to the marked suppression caused by methylmercury (MeHg). A key biochemical response to cadmium exposure is the induction of intracellular protective mechanisms. Fortier *et al*. (2008) observed a significant increase in intracellular thiol levels (including glutathione, GSH) in both lymphocytes and monocytes at all tested concentrations of heavy metals, including Cd. This is a cellular defence mechanism against oxidative stress. Furthermore, metallothionein (MT) levels, which are cysteine-rich proteins crucial for metal detoxification, were significantly increased in lymphocytes exposed to lower concentrations of Cd, either alone or in mixtures (Fortier *et al*., 2008). This aligns with the understanding that Cd is a potent inducer of MT synthesis, which binds the metal and mitigates its toxicity.

In a larger human population study, Huang *et al*. (2022) provided clinical evidence linking cadmium exposure to specific hematological changes. Their cross-sectional analysis of 2,447 participants found that higher urinary cadmium levels were significantly associated with an elevated eosinophil count, even after adjusting for multiple confounders. This suggests a pro-inflammatory or allergic-type response elicited by cadmium exposure. The study did not, however, find a significant independent association between urinary Cd and total white blood cell (WBC) count in the multivariable model (Huang *et al*., 2022). The immunomodulatory effects of cadmium appear to be dose-dependent and cell-type specific. While it may not be overtly cytotoxic at low, physiological concentrations, it can disrupt immune competence by suppressing lymphocyte proliferation and NK cell activity in some scenarios, while simultaneously provoking an eosinophilic response and inducing protective biochemical pathways like thiol and MT production in others. This duality underscores the complexity of cadmium's hematological impact, which can range from immunosuppression to the promotion of specific inflammatory responses.

2.8 PHYTOWASTE AND THEIR ROLE AS NUTRACEUTICALS

Parallel evolutionary processes are said to be the cause of the well-documented interdependence and relatedness of living things. (Shil *et al*., 2014). Due to their traditional use as food and medicine, plants are used in ethnomedicine to treat and cure a variety of illnesses, diseases, and poisonings, as well as to reverse or lessen their toxicity. Since the outer skins of these plant produce are typically inedible and deemed useless, they are frequently peeled off and thrown away. The hard, previously inedible seeds and peels are frequently considered phytowastes and thrown away as such. Street and market fruit vendors, as well as the fruit processing industries, produce a lot of trash, which, if not managed and used appropriately, could be harmful to the environment and cause illness. Reusing them as a different source of antioxidants could therefore help reduce pollution and provide the pharmaceutical and nutraceutical industries with quantifiable economic profits as well as affordable new generational therapies (Duda-Chodak and Tarko, 2007). The same beneficial ingredients that are often present in fruit may also be present in fruit peel trash. These essential ingredients can be combined to create formulations with nutritional, energetic, and pharmacologic/medical benefits. Fruit peel trash recycling has not only reduced solid waste issues but also aided in the discovery of significant compounds with demonstrated critical applications. Through appropriate distillation, industrial extraction, scientific incorporation, and management of these fruit peels, phytochemicals of interest can also be isolated (Wolfe and Liu, 2003). In order to isolate and extract secondary metabolites, fruit peel extracts are currently one of the main sources (Altemimi *et al*., 2017; Rafiq *et al*., 2018; Sharma and Akansha, 2018). Aside from their therapeutic and advantageous effects, which have been scientifically demonstrated and recorded through numerous in vitro, in vivo, and clinical phase trials conducted across various cultures and civilizations, they have been shown to have positive effects on a wide range of illnesses, such as cancer, diabetes, cardiovascular diseases, and osteoarthritis. The abundance of bioactive substances, including gallic acid derivatives, phenolic acids, gallic acid flavonoids, catechin, and mangiferin, is the reason for this.

2.9 WATERMELON (*Citrullus lanatus*)

Snacking has become much more popular in recent years. However, because of the high prevalence of noncommunicable diseases like cancer, diabetes, hypertension, and cardiovascular diseases (CVD), consumers are more concerned about their health than they were in previous years (Choudhary *et al*., 2015) Their understanding of food has therefore evolved from being primarily influenced by flavor and appearance to taking into account the idea of optimal nutrition by avoiding foods linked to nutritional deficiency. There is interest in substituting antioxidant-rich fruits for daily energy-dense snacks as a result of the recent dietary guideline to increase intake of foods high in natural antioxidants (Elumalai *et al*., 2013). In order to meet customer demands, this tendency also puts pressure on the food industry to produce wholesome food, ideally from natural sources. Customers are urged to nibble on natural goods like fruits and vegetables in this situation. The exotic staple fruit known as watermelon (*Citrullus lanatus*) is said to have health benefits for humans due to its phytochemicals and minerals (Choudhary *et al*., 2015).

In addition to minerals including calcium, magnesium, iron, and phosphorus, it is a good source of vitamins B, C, and E. It has antioxidants with anti-inflammatory and antihypertensive qualities, as well as a protective effect against toxicity caused by carbon tetrachloride, according to epidemiological research (Ijah *et al*., 2015). According to published research, natural phytochemicals like lycopene, polyphenols, vitamin C, and β-carotene exert their effects through a variety of pathways, including immune system response, cell growth regulation, and gene expression modification (Ijah *et al*., 2015).

2.9.1 BACKGROUND OF WATERMELON

A native of tropical regions of Africa close to the Kalahari Desert, watermelon belongs to the Cucurbitaceae family (Naz *et al*., 2014). It is referred to by botanists as a "pepo," a fruit with a meaty interior and a thick exterior (Mehra *et al*., 2015). It is a popular summer fruit that people love for its refreshing properties, appealing color, delicate flavor, and high water content, which helps to alleviate summertime thirst (Romdhane *et al*., 2017). Oberio and Sogi (2017) state that watermelon fruits produce 10.4% pomace, 31.5% rind, and 55.3% juice. The orange and red hues of watermelon are caused by carotenoids like β-carotene and lycopene, respectively. The sweetness of watermelon is mainly due to a combination of sucrose, glucose, and fructose. Sucrose and glucose account for 20–40% and fructose for 30–50% of total sugars in a ripe watermelon (Elumalai *et al*., 2013).

Plate 2.3: Watermelon (Elumalai *et al*., 2013)

2.9.2 PHYTOCHEMICAL PROPERTIES OF WATERMELON

2.9.2.1 LYCOPENE

The most unsaturated carotenoid is lycopene ($C_{40}H_{56}$), a straight-chain hydrocarbon with 13 double bonds, 11 of which are conjugated (Kehili *et al*., 2017). Whereas lycopene in human plasma is an isomeric mixture with 50% of the total lycopene as cis isomers, lycopene is found in natural sources in the trans form (Elumalai *et al*., 2013). It is evident as a red pigment that contributes to the appealing color of tomatoes, watermelon, guava, and red bell peppers. Lycopene accumulates in human tissue and makes up between 21 and 43 percent of all carotenoids (Elumalai *et al*., 2013).

Vegetables and fruits provide lycopene (Castro-López *et al*., 2017). As a result, the body absorbs it through food. About 10–30% of lycopene is thought to be absorbed by the body, and the average daily consumption in developed nations is 5–7 mg. Following ingestion, lycopene undergoes digestion in the stomach, where it transforms into a lipid phase that is released by pancreatic lipases and bile salts (Petyaev, 2016). Through passive transport and the use of a transporter, the scavenger receptor class B type 1 protein formed liposomes are taken up by the intestinal walls. It is then transferred as chylomicrons to fatty tissues and organs such the liver, testes, seminal vesicles, and adrenal glands after being further integrated into lipid micelles in the small intestine (Elumalai *et al*., 2013). Compared to the juice, watermelon pomace is said to be a more rich source of lycopene. Watermelon pomace was 20–24 mg/100 g, according to Oberoi and Sogi (2017). The portion of watermelon with the highest concentration of lycopene is still being investigated. Watermelon's lycopene content varies depending on the cultivar and growing environment. Because of its purported health benefits, lycopene has drawn the attention of food and health researchers both (Oberoi and Sogi, 2017). A watermelon's lycopene to carotene ratio is higher (1:12), which results in a remarkable antioxidant potential (Naz *et al*., 2014). It has a great deal of promise for use in the prevention and treatment of certain chronic illnesses. Foods high in lycopene are referred to as functional foods based on this particular feature (Soteriou *et al*., 2014).

2.9.2.2 β-CAROTENE

β-carotene is an insoluble vitamin and lipophilic macronutrient that contains retinoic, retinal, and retinol, among other unsaturated nutritious organic compounds. It has two unconjugated and eleven conjugated double bonds among its 40 carbon atoms (Kulczynski *et al*., 2017). Because of its lengthy, highly conjugated chain, β-carotene is exceedingly insoluble in a variety of solvents, including ethanol and water. It functions as a precursor for vitamin A in the human body and is visible as the orange hue of fruits and vegetables. According to Lin *et al*. (2018), intestinal epithelial cells directly absorb β-carotene in the intestine. Through photo-induced and heat isomerization, β-carotene is converted from its trans configuration form to cis isomers. Since the body is unable to produce β-carotene, it is mostly obtained from plant-based foods including carrots, sweet potatoes, spinach, melons, and mangos, according to Chen *et al*. (2017). β-carotene has the potential to be used as a functional food ingredient, but its absorption is less than 10% and it is not very bioavailable (Lin *et al*., 2018). There are significant amounts of β-carotene in watermelon. According to Kim *et al*. (2014), fresh watermelon flesh has 4.82 mg/g of β-carotene. As with other phytochemicals, the amount of β-carotene varies according to cultivar type and environmental circumstances.

It is capable of displaying both pro-oxidant and antioxidant qualities (Chen *et al*., 2017). These characteristics give β-carotene a favorable ability to inactivate specific ROS and a neuroprotective impact that protects against HDL and LDL (Rajabi *et al*., 2017).

2.9.2.3 VITAMIN C

The standard term for all chemical molecules displaying ascorbic acid's biological action is vitamin C ($C_6H_8O_6$) (Doll and Ricou, 2013). Ascorbate and dehydroascorbic acid are its two primary constituents (Pacier and Martirosyan, 2015) Pacier and Martirosyan (2015) also stated that in the 1920s, Hungarian biochemist Albert Szent-Györgyi discovered vitamin C in citrus fruit, vegetables, and adrenal glands as hexuronic acid. Because it is a vital ingredient that the body cannot produce on its own, it must be obtained from diet (Oberoi and Sogi, 2015) Water-soluble and vital, vitamin C is commonly added to a wide range of food products for nutrient enhancement and supplementation. It is crucial for the manufacture of collagen and certain hormones (Rodriguez-Roque *et al*., 2015). It has been determined that watermelon is a good source of vitamin C. Oberio and Sogi (2015) state that 3.72 mg/100 g of fresh watermelon juice is present. Similar to other criteria, variations in watermelon cultivars and environmental conditions cause variations in vitamin C. 20% of the recommended daily intake of vitamin C can be found in one cup of watermelon juice. According to Pacier and Martirosyan (Pacier *et al*., 2015), scurvy and nutritional deficiencies can be avoided with a daily intake of at least 10 mg. However, for best results, 90–500 mg per day is advised.

Through a number of possible processes, vitamin C may help cancer patients live better lives. Cancer patients typically experience high levels of oxidative stress and vitamin C deficiency (Takahashi *et al*., 2012) Consequently, taking this vitamin orally from natural sources may help individuals who suffer from chronic vitamin C insufficiency with their weariness and other symptoms. However, each patient experiences cancer pain differently, thus the types of tumors require different amounts of vitamin C. Additionally, vitamin C may be able to stop the blood flow to developing malignancies, which would stop the formation of cancer cells (Lemos *et al*., 2017) The other explanation is that vitamin C has anticancer qualities (Ijah *et al*., 2015), therefore depending on blood concentrations, eating foods high in vitamin C may help patients' cancer cells by producing pro-oxidant activity (Romdhane *et al*., 2017).

2.9.2.4 CITRULLINE

Watermelon is said to contain a lot of citrulline, a non-protein amino acid, according to Sonteriou *et al*. (2014). The researchers also found that citrulline is a metabolic step in the nitric oxide cycle and an efficient precursor for arginine. As a byproduct of the nitric oxide cycle, it has become a significant amino acid. Citrulline is thought to be a strong osmolyte and radical scavenger that protects against salt and drought stress (Kyriacou *et al*., 2018). According to Hong *et al*. (2015), watermelons contain the highest concentration of citrulline, with levels ranging from 0.7 to 3.6 mg/g fresh. Kyriacou *et al*. (2018), reported that a recent study done on 56 cultivars identified the mean value of citrulline as 3.1 mg g^{-1} that shows no link with cultivar type. Research is still ongoing to discover which section of the watermelon contains more citrulline than the other (meat, juice, and rind). According to Odewunmi *et al*. (2015), citrulline is primarily absorbed in the colon and is created spontaneously in the body by an enzyme reaction of nitrogen-carbon-containing L-glutamine. It's possible that citrulline from a natural source is more bioavailable than that from a synthetic one. According to a cohort research, people who consistently drank large amounts of watermelon juice with each of their three meals had higher levels of ornithine and arginine in their plasma than subjects who did not consume watermelon (Sai'd, 2014). These findings show that consuming citrulline from watermelon juice can raise the plasma content of arginine. Citrulline is a potent antioxidant and an effective hydroxyl radical scavenger, and its consumption is linked to a number of health advantages (Soteriou *et al*., 2014).

In fields including pharmacology, neurology, immunology, and skeletal anatomy, citrulline was proven to be effective (Odewunmi *et al*., 2015). Although the precise mechanism underlying its effects is yet unknown, it has been found to enhance erectile function and sexual stamina (Soteriou *et al*., 2014). For young people who have experienced trauma, burn injuries, extensive small bowel resection, or renal failure, it is crucial as an amino acid. Additionally, it has been shown to play a significant role in preventing anemia (Choudhary *et al*., 2014).

2.9.3 PHARMACOLOGICAL PROPERTIES OF *Citrullus lanatus*

2.9.3.1 ANTIOXIDANT ACTIVITY

The fruit and seeds of *Citrullus lanatus* are rich in antioxidants, which are essential for combating oxidative stress linked to chronic conditions like cancer, cardiovascular disease, and diabetes (Rahman *et al*., 2013). A prominent antioxidant is lycopene, a carotenoid that gives the fruit its red colour. Research indicates that lycopene can promote skin health and offer protection against skin cancer by blocking the harmful effects of UV radiation (Rao and Rao, 2007).

2.9.3.2 ANTI-UROLITHIATIC AND DIURETIC ACTIVITY

Research indicates that the pulp extract of watermelon can prevent the formation of kidney stones and promote urination. Studies in Wistar rats have shown that the extract reduces the formation and count of calcium oxalate crystals in the kidneys and urine, while also increasing urinary pH and output (Siddiqui *et al*., 2018). Furthermore, the extract was found to lower urea and creatinine levels in urine and correct imbalances of phosphate, calcium oxalate, and citrate. It also enhances kidney filtration, leading to decreased serum chloride and increased excretion of sodium and chloride (Byer and Khan, 2005).

2.9.3.3 ANTIMICROBIAL ACTIVITY

Various extracts from watermelon fruit and seeds contain bioactive compounds like tannins, flavonoids, and alkaloids, which demonstrate effectiveness against a range of bacteria and fungi (Thirunavukkarasu *et al*., 2010). These extracts have been shown to inhibit the growth of gram-negative and gram-positive bacteria, including *E. coli* and *Bacillus subtilis*, as well as fungal strains like *Candida albicans* and *Aspergillus niger*. Methanolic seed extract, in particular, has shown significant antimicrobial activity against pathogens such as *Vibrio cholerae* and *Shigella dysenteriae* (Sathya and Shoba, 2014).

2.9.3.4 ANTIDEPRESSANT ACTIVITY

In contrast to synthetic antidepressants, which can have various side effects (Beeder and Samplaski, 2020; Kapur *et al*., 2001), extracts from watermelon offer a natural alternative. Aqueous pulp juice and hexane seed extracts have demonstrated antidepressant and anti-anxiety effects in behavioural tests on rats, comparable to the drug fluoxetine but without causing complications in skeletal muscles (Rahman *et al*., 2013; Sandhya *et al*., 2020). The antidepressant effect is partly attributed to lycopene and other compounds like polyphenols and flavonoids, which help restore antioxidant enzyme levels and reduce oxidative stress associated with depression (Bose and Agrawal, 2007; Naz *et al*., 2014).

2.9.3.5 ANTI-TOXIC ACTIVITY AGAINST HEAVY METALS

Exposure to heavy metals like lead (Pb) is a known cause of reproductive dysfunction (Massányi *et al*., 2020). Methanolic extract of watermelon seeds has shown a protective effect against lead acetate-induced testicular toxicity in rats. Treatment with the extract improved testicular structure, increased sperm motility, viability, and count, and raised testosterone levels while reducing sperm abnormalities (Godspower *et al*., 2015).

2.9.3.6 FERTILITY ENHANCER

Compounds in watermelon, such as saponins, citrulline, and arginine, have been identified as beneficial for managing infertility (Gauthaman and Ganesan, 2008; Sandroni, 2001). Citrulline and arginine help improve erectile function by increasing nitric oxide concentration, which upregulates guanosine monophosphate to facilitate penile erection (Davies, 2015; Drewes *et al*., 2003). Ethanolic seed extract has been shown to enhance male sexual behaviour and increase levels of testosterone and luteinizing hormone, improving the structure of the testes (Onyinye and Emeka, 2019). The fruit also supports female fertility by boosting follicular stimulating hormone levels, which helps maintain pregnancy (Chike *et al*., 2011).

2.9.3.7 ANTI-ULCER AND GASTRO-PROTECTIVE ACTIVITIES

Methanolic seed extract of watermelon has demonstrated significant anti-ulcer properties in rat models, reducing gastric volume, free acidity, and total acidity (Bhardwaj *et al*., 2012). The pulp and rind extracts also protect the gastric mucosa, likely due to their content of flavonoids and polyphenols, which increase gastric pH and reduce ulcer severity (Kolawole and Dapper, 1996; Sharma *et al*., 2014).

2.9.3.8 HEPATO-PROTECTIVE ACTIVITY

Watermelon seed oil offers protection against liver damage. In studies where hepatotoxicity was induced in rats, the seed extract effectively restored normal levels of liver enzymes (ALT, AST, ALP) and improved the histological appearance of the liver (Madhavi *et al*., 2012).

2.9.3.9 LAXATIVE ACTIVITY

Consistent with its traditional use for constipation, the fruit pulp extract of watermelon has been found to enhance intestinal motility and increase faecal matter weight in rats, confirming its laxative properties (Sharma *et al*., 2011).

2.9.3.10 ANTI-GIARDIAL ACTIVITY

Certain extracts from watermelon fruit pulp, including those containing Cucurbitacin E and Cucurbitacin L 2-O-β-glucoside, have shown potent activity against *Giardia lamblia* trophozoites, suggesting a natural alternative to synthetic anti-giardial drugs (Loly *et al*., 2011).

2.9.3.11 ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

Watermelon possesses notable pain-relieving and anti-inflammatory properties. Aqueous peel extract and methanolic seed extract have demonstrated analgesic effects in various pain models, as well as anti-inflammatory activity in tests for edema (Deng *et al*., 2010; Gill *et al*., 2010; Kumari *et al*., 2013). These extracts also exhibit free radical scavenging capabilities.

2.9.3.12 ANTI-ATHEROSCLEROTIC ACTIVITY

Consumption of watermelon juice is beneficial for cardiovascular health. The extract of the fruit, rich in citrulline, has been shown to reduce atherosclerosis in mice by decreasing aortic thickening, improving blood vessel relaxation, balancing inflammatory cytokines, and lowering plasma cholesterol (Poduri *et al*., 2013).

2.9.3.13 ANTI-DIABETIC ACTIVITY

Methanolic seed extract of watermelon has anti-diabetic effects, significantly reducing blood glucose levels in diabetic rats (Omigie and Agoreyo, 2014). This activity is attributed to constituents like tannins, saponins, and soluble fibres. Flavonoids in the seeds are also thought to stimulate insulin secretion from pancreatic beta cells (Mahesh and Menon, 2004).

2.9.3.14 ANTI-ASTHMATIC AND ANTI-DIARRHEAL ACTIVITIES

Ethanolic seed extract has demonstrated both anti-asthmatic and anti-diarrheal activities. These effects are believed to result from the extract's ability to modulate contractile responses in tissues through calcium-mediated signalling pathways (Wahid and Saqib, 2022).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 MATERIALS

3.2 CHEMICALS, SOLVENTS AND REAGENTS

Absolute ethanol, normal saline, Chloroform, distilled water, Cadmium chloride, Em Vit vitamin C.

3.3 APPARATUS, MATERIALS AND EQUIPMENTS

Plain bottles, EDTA bottles, cotton wool, analytical weighing balance, Handgloves, Soxhlet extractor apparatus, Whatmann paper, filter cloth, oral gastric tube, Syringes, Spatula, Beakers, Measuring cylinders, foil paper, Dissecting kits, Surgical blades, Bulk scientific atomic absorption spectrophotometer with (VGP 210).

3.4 PROCUREMENT OF PLANT AND PREPARATION OF PLANT EXTRACTS

Rinds of *Citrullus lanatus* were obtained from New Benin market at Oredo local government area in Benin city. The full bag of *Citrullus lanatus was peeled* and the rinds were kept aside. Afterwards, the rinds were dried in a dehydrator at a controlled temperature (37 degree Celsius). The dried samples were then blended into powder with the aid of an industrial blender. The samples were weighed and placed inside a thimble for soxhlet extraction. After extraction, the liquid was filtered using whatmann paper. The filtrate was then concentrated using crude method to get a semi solid form. It was dispensed in a small sample container and labelled and stored in a refrigerator.

3.5 EXPERIMENTAL ANIMALS

Twenty five Wistar rats weighing 80 g to 150 g were obtained from Ibadan and were appropriately housed in the Faculty of Science Laboratory technology, University of Benin. Appropriate ventilation, feed and lighting were provided for them. They were acclimatized for 7 days before experiment.

3.6 EXPERIMENTAL DESIGN

Group 1 served as the control and was administered 1 ml of distilled water

Group 2 served as the negative control and was administered Cadmium chloride (15 mg/kg)

Group 3 was administered 50 mg/kg of Vitamin C

Group 4 was administered 250 mg/kg of the extract (*Citrullus lanatus*)

Group 5 was administered 500 mg/kg of the extract (*Citrullus lanatus*)

The rats used for the study were maintained based on the guild lines of the national institute 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 approval number UNIBEN/FSLT/00031

3.7 COLLECTION OF ORGANS AND BLOOD

60 days after administration, the animals were sacrificed. They were euthanized using an analytical chloroform and their blood was collected through the abdominal aorta using a 5ml syringe, and put in a plain bottle and EDTA bottle and prepped for hematology analysis.

3.8 HEMATOLOGY ASSAY

Blood samples were immediately analyzed for full blood count parameters using an automated haematology analyzer (ERMA PCE-210N). All procedures, including equipment calibration, standardization, and sample analysis, adhered strictly to the manufacturer's instructions. The analyser utilizes the principle of electrical impedance to count and size blood cells. When a diluted blood sample is passed through a small aperture, individual cells disrupt the flow of current, generating electrical pulses. The size and amplitude of these pulses are proportional to the size of the cells. The instrument amplifies these signals, removes background noise, and differentiates between the various blood cells using specialized circuits. Advanced algorithms analyze the signals and generate histograms to determine cell counts and size distributions. Platelet counting employs sophisticated techniques to minimize interference from RBCs, which can produce similar electrical signals.

After thoroughly mixing the sample, a small amount (20 µL) of whole blood was taken. The analysis was then run immediately, with results appearing on the screen within 1-2 minutes and then printed out.

3.9 HEAVY METALS ANALYSIS (CADMIUM)

The blood samples were homogenized to ensure uniformity. 0.5 ml of blood was transferred into a pre-cleaned digestion tube. 10 ml of the mixed acid (Nitric-Pechloric-sulphuric acid- 5:2:1) was added. The digested tube was brought to the heater and heated till a clean solution was gotten (disregard white fume and continue heating). The solution was removed from the heater, allowed to cool and a little amount of deionized water was added. The solution was filtered with a whatman filter paper into a 100 ml volumetric flask and the volume made up to 100 ml using the deionized water. The reagent blank was prepared and the metals of interest were determined with the Atomic absorption spectrophotometer.

3.10 STATISTICAL ANALYSIS

Data was subjected to one way analysis of variance (ANOVA) and Tukey's multiple comparison test of significant difference ($p < 0.05$) and represented with mean and standard error of mean. The statistical package of social sciences (SPSS) version 19 was used for analysis.

CHAPTER FOUR

RESULTS

Table 4.1: Effect of the hydroethanolic extract of watermelon rind on the initial and final body weight

GROUPS	INITIAL WEIGHT	FINAL WEIGHT	% WEIGHT CHANGE
Control	155.50 ± 6.20	228.50 ± 9.75	47.31 %
Negative control (15mg/kg) $CaCl_2$	177.75 ± 17.24	238.00 ± 26.11	37.41 %
Vitamin C (50mg/kg)	140.25 ± 8.96	253.75 ± 19.03	85.53%
250mg/kg *Citrullus lanatus*	131.25 ± 3.50	241.25 ± 19.72	83.66 %
500mg/kg *Citrullus lanatus*	112.67 ± 4.48	201.67 ± 15.90	84.66 %

Table 4.1: Initial weight and effect of the extract on the final body weight of the rat after 60 days. The Negative control group administered cadmium showed the lowest percentage weight change (37.41%). The extract group (500mg/kg), had the highest percentage weight increase (84.66 %), followed by the 250mg/kg (83.66 %) extract group and Vitamin C (83.53%), and control group (47.31%) group respectively

Table 4.2: Effect of Hydroethanolic extract of *Citrullus lanatus* on the Cadmium level in the blood

GROUPS	BLOOD
Control	0.13 ± 0.04
Negative control (15mg/kg) $CaCl_2$	0.21 ± 0.02
Vitamin C (50mg/kg)	0.17 ± 0.01
250mg/kg *Citrullus lanatus*	0.14 ± 0.05
500mg/kg *Citrullus lanatus*	0.32 ± 0.08

Data were represented as mean ± SEM. a represent a significant difference from control group, b represent a significant difference from Negative control group, c represent a significant difference from vitamin C group, d represent a significant difference from 250mg/kg extract group, e represent a significant difference from 500mg/kg extract group. No letter represent no significant difference from any group. There was no significant difference between the groups.

Table 4.3: Effect of *Citrullus lanatus* rinds on the hematological parameters of Wistar rats

GROUPS	WBC	LYM	MID	GRAN	RBC	HGB	HCT	PLT
Control	2.43 ± 0.62	78.03 ± 2.10	12.50 ± 2.61	9.48 ± 1.44	8.01 ± 0.52	14.78 ± 0.94	45.00 ± 3.60	550.75 ± 90.41
Negative control (15mg/kg) $CaCl_2$	3.68 ± 0.53	86.08 ± 2.51	7.43 ± 1.23	6.50 ± 1.33	8.17 ± 0.41	15.23 ± 0.73	47.20 ± 3.36	439.75 ± 142.07
Vitamin C (50mg/kg)	5.13 ± 1.03	82.28 ± 5.12	10.55 ± 3.55	6.98 ± 1.68	7.58 ± 0.96	13.85 ± 2.08	43.75 ± 6.44	766.25 ± 71.84
250mg/kg *Citrullus lanatus*	4.60 ± 1.03	79.13 ± 6.03	11.40 ± 2.22	9.43 ± 3.91	8.01 ± 0.30	14.33 ± 0.68	47.08 ± 1.24	766.50 ± 85.75
500mg/kg *Citrullus lanatus*	4.37 ± 0.64	87.77 ± 3.49	6.47 ± 2.01	5.77 ± 1.93	7.46 ± 0.14	13.40 ± 0.38	42.57 ± 1.72	732.33 ± 130.51

Data were represented as mean ± SEM. a represent a significant difference from control group, b represent a significant difference from Negative control group, c represent a significant difference from vitamin C group, d represent a significant difference from 250mg/kg extract group, e represent a significant difference from 500mg/kg extract group. No letter represent no significant difference from any group. There was no significant difference between the groups

WBC: White blood cells, RBC: Red blood cells, LYM: Lymphocytes, GRAN: Granulocytes, MID: combined value of other types of white blood cells not classified as lymphocytes or granulocytes, HGB: haemoglobin, HCT: haematocrit, PLT- platel

CHAPTER FIVE

DISCUSSION

The investigation of body weight change serves as an important Biomarker of the overall health and metabolic status of experimental animals. In this study, the group administered cadmium (the negative control) showed the lowest percentage weight gain (37.41%) over the 60-day period. This aligns with the established pathophysiology of cadmium toxicity. Cadmium is a potent toxicant known to induce multi-organ damage, particularly in the liver and kidneys, which are central to nutrient metabolism and detoxification (Satarug, 2018; Tinkov *et al.*, 2018). The observed stunted growth and reduced weight gain can be due to cadmium-induced anorexia, gastrointestinal distress, and impaired nutrient absorption. Furthermore, cadmium exposure promotes oxidative stress, which can damage cellular structures and disrupt normal metabolic processes (Matović *et al.*, 2015). In contrast, the groups treated with the hydroethanolic extract of *Citrullus lanatus* rind showed remarkable and dose-dependent improvements in body weight. The group receiving 500 mg/kg of the extract demonstrated the highest percentage weight increase (84.66%), closely followed by the 250 mg/kg group (83.66%) and the Vitamin C group (83.53%). The performance of the extract groups was comparable to the Vitamin C group, a known antioxidant standard. This suggests that the watermelon rind extract possesses significant protective and restorative properties against cadmium-induced toxicity. The therapeutic effect is likely mediated by the rich profile of bioactive compounds present in the rind, including lycopene, β-carotene, vitamin C, and citrulline (Elumalai *et al.*, 2013; Naz *et al.*, 2014). These compounds are potent antioxidants that can scavenge the reactive oxygen species (ROS) generated by cadmium, thereby reducing oxidative damage to cellular membranes and organelles. By mitigating this damage, the extract likely helped preserve the structural and functional integrity of the gastrointestinal tract, liver, and kidneys, facilitating better nutrient absorption and utilization, which in turn supported normal growth and weight gain. The findings are consistent with studies highlighting the antioxidant and anti-inflammatory potential of watermelon phytochemicals in countering various toxic insults (Rahman *et al.*, 2013; Godspower *et al.*, 2015).

The level of cadmium concentration in the blood (Table 4.1) revealed that while the negative control group had the highest mean cadmium level (0.21 ± 0.02 µg/dL), there was no statistically significant difference ($P > 0.05$) between any of the experimental groups. The group treated with the high dose of the extract (500 mg/kg) showed a numerically higher mean cadmium level (0.32 ± 0.08 µg/dL) than the negative control, though this was also not statistically significant. The absence of a significant reduction in blood cadmium levels in the treatment groups, particularly the 500 mg/kg extract group, presents a complex outcome. Cadmium's toxicokinetics are characterized by its rapid clearance from the bloodstream and subsequent accumulation in soft tissues, primarily the liver and kidneys, where it binds strongly to metallothioneins (MTs) (Sabolic *et al.*, 2010; Satarug, 2018). Blood cadmium is a marker of recent exposure, while urine cadmium reflects body burden. The primary mechanism of action for many chelating agents or detoxifying extracts is not necessarily to rapidly mobilize cadmium from deep tissue stores into the blood for excretion, but rather to prevent its initial absorption and/or mitigate its toxic effects at the cellular level. The increase in the 500 mg/kg group could be interpreted in two ways. Firstly, it may indicate a limited mobilization of cadmium from tissue stores back into the bloodstream. However, a more plausible explanation, supported by the hematological and body weight data, is that the extract's primary role was one of cytoprotection rather than chelation. The bioactive compounds in *Citrullus lanatus*, such as polyphenols and flavonoids, are known for their potent antioxidant and metal-chelating properties (Brzóska *et al.*, 2016). They may form complexes with cadmium in the gut, reducing its bioavailability, or they may upregulate endogenous protective systems like glutathione and metallothioneins within cells, thereby sequestering the metal and rendering it less toxic without altering its overall distribution significantly (Wang *et al.*, 2013).

The evaluation of hematological parameters provides information into the functional state of the bone marrow and the immune system, both of which are targets for heavy metal toxicity. The results (Table 4.2) indicate that cadmium exposure did not cause statistically significant alterations in most hematological indices compared to the control ($P > 0.05$). The white blood cell (WBC) count showed a trend of increase in the cadmium-only group and the treatment groups compared to the control, though not significant ($P > 0.05$). This could suggest a mild, compensatory inflammatory or immune response triggered by cadmium. Cadmium has been shown to have immunomodulatory effects, which can be complex and dose-dependent; it can be immunosuppressive at high doses but may provoke inflammatory responses at lower, sub-chronic exposure levels (Fortier *et al.*, 2008). The lymphocyte (LYM) count was highest in the cadmium and 500 mg/kg extract groups. While cadmium is known to inhibit lymphocyte proliferation in some *in vitro* studies (Borella *et al.*, 1990), *in vivo* exposure can sometimes lead to lymphocytosis as part of a stress or inflammatory response. The extract did not normalize this trend but may have supported overall immune cell viability. The negative control group had the lowest mean platelet count (439.75 ± 142.07), while all treatment groups, especially Vitamin C (766.25 ± 71.84) and the 250 mg/kg extract (766.50 ± 85.75), showed markedly higher counts.

Although not statistically significant due to high variability. Thrombocytopenia can be a consequence of cadmium toxicity, as the metal can induce oxidative damage to bone marrow megakaryocytes, the precursor cells for platelets. The restoration of platelet counts in the treatment groups strongly indicates a protective effect of both Vitamin C and the watermelon rind extract on the hemopoietic system. This aligns with the known role of antioxidants in supporting bone marrow function and mitigating oxidative stress-induced cytopenia (El-Boshy *et al.*, 2015). The antioxidants in the extract, particularly vitamin C and lycopene, likely helped protect these sensitive progenitor cells from cadmium-induced oxidative apoptosis, thereby maintaining normal platelet production.

The red blood cell indices (RBC, HGB, HCT) remained largely stable across all groups, showing that the duration or dose of cadmium exposure was not sufficient to induce overt anemia.

CONCLUSION

This research demonstrates that the hydroethanolic extract of *Citrullus lanatus* (watermelon) rind possess protective effect against cadmium-induced physiological and hematological disturbances in Wistar rats. The extract countered the stunted growth caused by cadmium toxicity, promoting body weight gain to a level comparable with the standard antioxidant, Vitamin C. While the extract did not significantly reduce blood cadmium concentration, its most notable property was in mitigating cadmium-induced damage to the hemopoietic system, as shown by the restoration of platelet counts. This cytoprotective effect is likely attributable to the rich profile of bioactive compounds in watermelon rind, such as lycopene, β-carotene, vitamin C, and citrulline, which function as potent antioxidants. Therefore, watermelon rind, an agricultural by-product, shows promise as a valuable, natural, and sustainable nutraceutical agent for ameliorating the toxic effects of heavy metals like cadmium.

LIMITATIONS

1. The study utilized a relatively small sample size and was conducted over a period of 60 days. A larger sample size and a longer duration might have provided more accurate result, and revealed more pronounced long-term effects of both cadmium toxicity and the extract's therapeutic properties.
2. The result that the blood cadmium level of the groups showed no significant difference creates ambiguity.

RECOMMENDATIONS

1. Larger sample size should be used for more accurate result
2. The number of days should be extended for further studies
3. Different doses of cadmium should be used for future research to help in identifying any possible dose response relationship

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APPENDIX

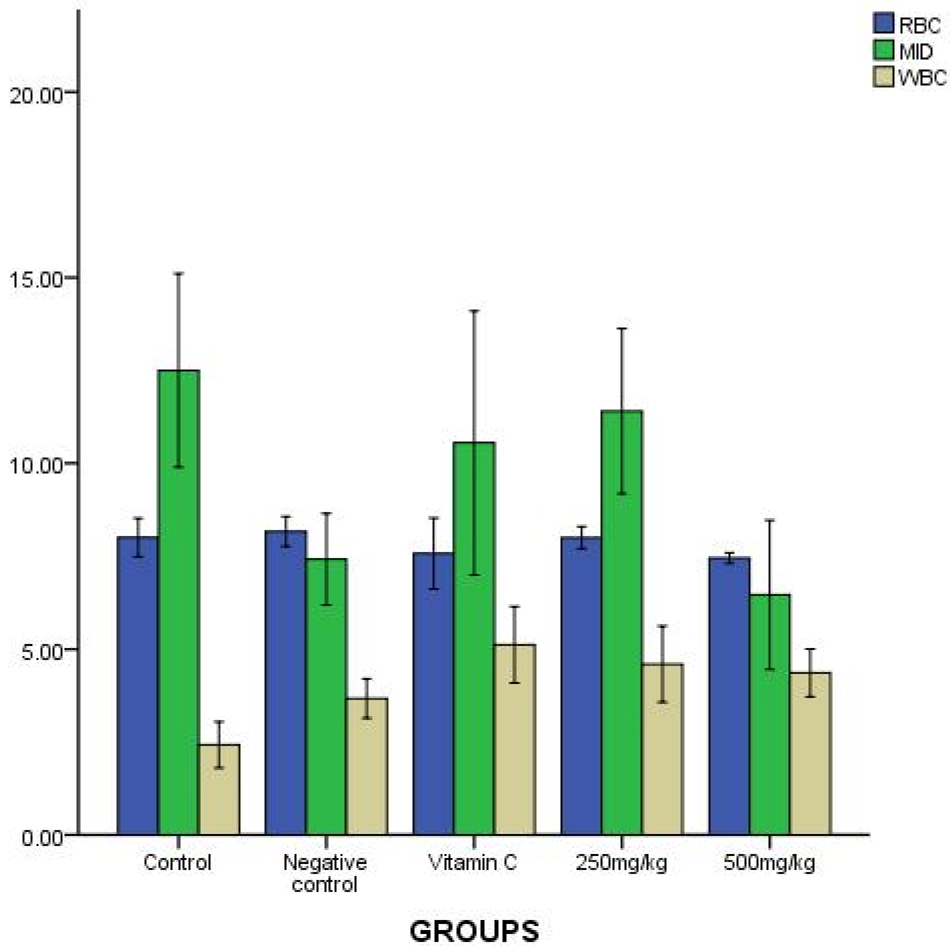

Figure 1: hematological parameters

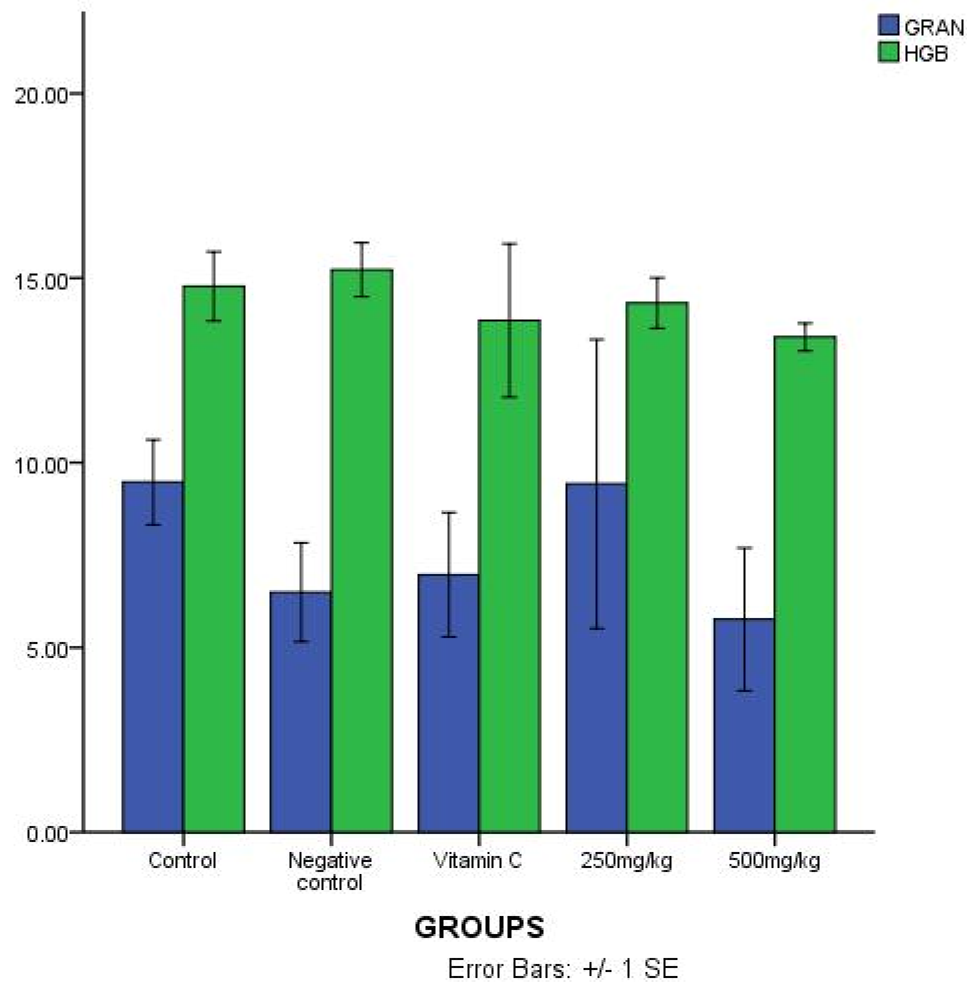

Figure 2: Hematological parameters

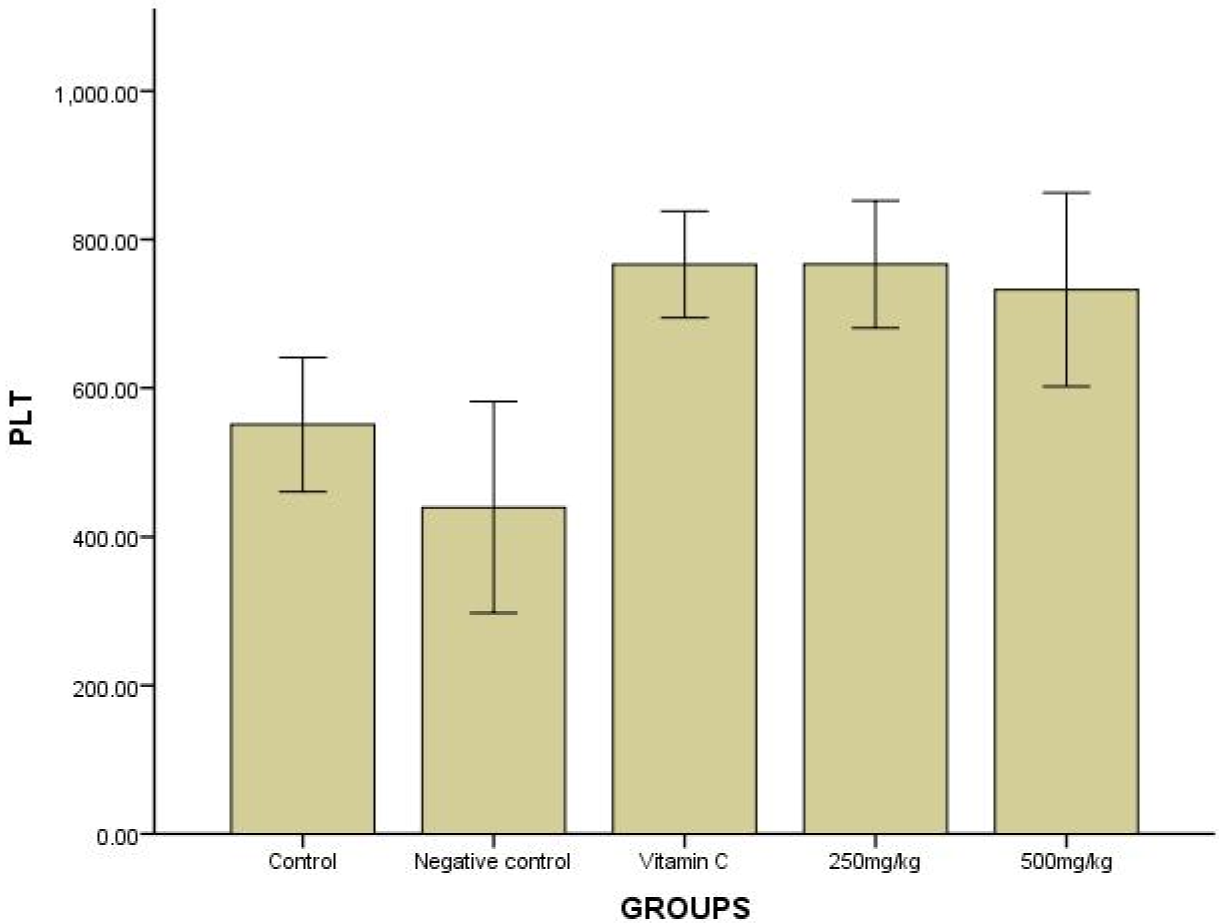

Figure 3: hematological parameter