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GC-MS Analysis and Antifungal Activity of *Morinda Lucida* Extract

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Abstract

The increasing climatic and environmental change and injudicious use of antibiotics has led recently to increase fungal infections resistance to available pharmaceutical antifungal drugs. Consequently, research for alternative local medicinal source of plant origin is on the increase, thus, the aim of this research work is to determine the chemical constituents and antifungal activity of Morindalucidaextracts. The Morindalucidaleaves were collected, dried under shade, pulverized and extracted with hexane, ethylacetate and methanol solvents using soxhlet extractor in order of increasing polarity. The three crude extracts were concentrated using rotary evaporator and the phytochemical screening was conducted according to standard methods while the antifungal analysis was conducted using the agar well diffusion method. Vacuum liquid chromatography was used for isolation of the oil from the hexane extract, which was characterized by gas chromatography-mass spectrometry (GC-MS). The result shows the presence of glycoside, saponins, phenolics, alkaloids, terpenoids, steroids, eugenols and flavonoids with the exception of tanins in all the extracts. The hexane, ethylacetate and methanol extracts of Morindalucidaexhibit moderate, strong, and very strong antifungal inhibitory activities respectively against the tested fungi strains of Candida auris, Candida albicans, Aspergillusfumigatus, Trichophytonmentagrophytes and Epidermophytonfloccosum. The GC-MS analysis of the hexane extractshows 48 compounds including hexadecanoic acid methyl ester, and squalene among others, which have reported antifungal activity. This study corroborates the use of the plants as antifungal agents by traditional people.

1. Introduction

Throughout history, plants and herbs have been vital for both nourishment and traditional healing practices, as well as for advancing modern medicine. Their ability to synthesize a broad spectrum of chemical compounds is essential for biological functions and defense mechanisms against threats such as insects, fungi, bacteria, viruses, and other pathogens [1]. Plants go beyond mere

sustenance; they serve as formidable allies in enhancing human health and well-being. Their wide range of nutrients, antioxidants, and bioactive compounds possess a lot of health benefits that bolster different bodily functions.

The avocado (*Persea americana*), a member of the Lauraceae family, is celebrated for its delectable fruit. Originating from the Western Hemisphere, spanning from Mexico to the Andean regions, avocados flourish in warm climates and are widely cultivated. Their fruits display greenish or yellowish flesh, characterized by a velvety texture and a luxuriously rich, nutty taste. Packed with essential nutrients like thiamine, riboflavin, and vitamin A, avocados also boast up to 25 percent unsaturated oil content in certain varieties, further enhancing their nutritional prowess [2]. The avocado, characterized by its olive-green skin and rich, creamy yellow flesh packed with various fatty acids like linoleic, oleic, palmitic, stearic, linolenic, capric, and myristic acids, is a staple in human diets and serves medicinal purposes in Mexico and elsewhere [3]. Although the seed, which makes up 13-18% of the fruit, is often discarded during processing, this practice presents ecological concerns [4]. Nevertheless, it holds potential for industry applications due to its abundance of phytosterols, triterpenes, fatty acids, and two unique glucosides of abscisic acid [5].

There is a global trend towards industrial fruit processing, often resulting in the disposal of byproducts. However, these byproducts can lead to ecological issues, including an increase in insect and rodent populations. Therefore, there is a need for studies to explore the potential benefits of these byproducts as sources of food supplements or medicinal products. Various parts of the avocado pear have been traditionally used for different purposes, including its antimicrobial properties. Investigating the dietary and therapeutic potential of underutilized agro-food waste can also contribute to reducing environmental waste. The avocado seed is an example of an underutilized, non-edible part of the fruit, typically discarded as residue. Research on non-edible fruit parts is gaining prominence and holds promise for future profitability. This is primarily due to its significant reduction in waste production and the fact that non-edible parts, like the avocado seed, are rich in valuable bioactive compounds, especially natural antioxidants. Although the avocado seed is often considered waste during pulp processing, it holds potential ecological and industrial interest as a source of bioactive compounds. Its chemical composition includes phytosterols, fatty acids, triterpenes, and novel glucosides of abscisic acid. The avocado seed has demonstrated various biological activities, such as antioxidant, antihypertensive, fungicidal, larvicidal, hypolipidemic, and recently, amoebicidal and germicidal activities.

Cadmium (Cd) exposure in humans mainly arises from inhalation or ingestion. The absorption of inhaled cadmium dust varies from ten to fifty percent, contingent upon particle size, with minimal absorption through skin contact. Likewise, ingested cadmium, depending on particle size, is absorbed at a rate of approximately five to ten percent. Individuals with deficiencies in iron, calcium, or zinc tend to exhibit increased rates of intestinal absorption [6]. Smokers consistently exhibit higher levels of cadmium (Cd) in their blood and kidneys compared to nonsmokers. Inhalation of cadmium in occupational settings, such as welding or soldering, carries a significant risk and can potentially result in severe chemical pneumonitis [6]. The primary source of cadmium exposure is through consuming contaminated food items like crustaceans, organ meats, leafy vegetables, and rice from specific regions of Japan and China. Additionally, exposure can occur through water contaminated by old zinc/cadmium-sealed pipes or industrial pollution, leading to

potential long-term health consequences. Moreover, drugs and dietary supplements may also introduce cadmium contamination [7].

The bulk of cadmium (Cd) found in erythrocytes is linked to high-molecular-weight proteins, while a minor fraction is attached to hemoglobin [8]. Hematopoiesis, especially in conditions such as itai-itai disease, suffers adverse effects, resulting in severe anemia, frequently accompanied by notable suppression of erythropoietin production (9). Cadmium induces liver damage through oxidative stress, leading to lipid peroxidation, disruption of cellular membranes, and impairment of liver function. Additionally, cadmium can interfere with antioxidant defense mechanisms, exacerbating oxidative damage in the liver. In the kidneys, cadmium primarily accumulates in the proximal tubular cells, where it disrupts cellular functions and induces oxidative stress which can result in damage of the renal tubules and glomeruli, impairing kidney function. Cadmium also interferes with calcium metabolism and disrupts the balance of essential metals, further contributing to kidney damage. In this study, the chelating effect of *Persea americana* seed extract on Organ-Tissue parameters of wistar rats exposed to cadmium, was investigated.

2.0. Materials and Method

2.1 Chemical reagents

All the chemicals used in this study were of analytical grade. 100g Cadmium Chloride, 100g Ethylenediamine tetracetic acid (EDTA) and 90% alcohol manufactured by Cartivalue Chemical Limited, Mumbai, India were obtained from a chemical store in Benin City.

2.2. Extract preparation

Fresh *Persea americana* (Avocado pear) were obtained from a local market (Effurun market) in Uvwie Local Government of Delta state, Warri. The seeds of the fresh *Persea americana* were manually separated from its fruit washed with distilled water and air dried for about three weeks until they became dry. They were transferred to the incubator where they were incubated at 40-45°c for about two hours till they were dried. They were crushed into a coarse dust with mortar and pestle. They were ground in a mechanical grinder to fine dust. The ground seed was weighed. It was then extracted using 90% alcohol, it was soaked for 72 hours and agitated periodically. The mixture was filtered using cheesecloth and a Whatman filter paper no 1 and the shaft was discarded. The filtrate was collected and placed in a crucible and put in a water bath at a controlled temperature of 44^{o}C for 40mins. It was then removed and placed in a sterile bottle and placed in the refrigerator at a temperature of 4^{o}C.

2.3. Experimental animals and design

A total of thirty (30) healthy wistar rats (male and female) with weights ranging from 160g to 250g were purchased from the Pharmacology animal house, Faculty of Pharmacy, and housed in plastic cages in the Animal House of the Department of Animal Environmental Biology (AEB), University of Benin, Benin City and they were acclimatized for two weeks before the commencement of the experiments.

All animals were given food (rat chow – Vital Feeds) and water *ad libitum*, they were also exposed to 12 hours of light and 12 hours of darkness. Rats were weighed at the beginning, during, and at

the end of administration. The animals were handled according to the guidelines of the Institutional Animal Ethics Committee of the Department of Physiology, University of Benin.

After 14 days of acclimatization to laboratory conditions, the wistar albino rats were divided into five (5) groups of six (6) rats in each group.

Group I served as the negative control and was administered distilled water.

Group II served as the positive control and was administered cadmium chloride (8 mg/kg).

Group III was administered ethylenediaminetetraacetic acid (EDTA) and cadmium chloride (8 mg/kg)

Groups IV and V were administered ethanoic extract of *Persea americana* (250 mg/kg and 500 mg/kg, followed concomitantly by cadmium chloride (8 mg/kg) for 4 weeks.

All administrations were via the oral route.

2.4. Collection of blood and tissues for testing

After 28 days, the rats were fasted overnight and the final body weights of all the rats were taken. The animals were sacrificed and the abdominal cavity was carefully opened. The liver and kidney were excised and blood was collected from the abdominal aorta. The samples were labeled and taken to the laboratory for analysis.

2.5. Heavy metal analysis

The collected samples were decomposed by a wet chemical digestion method for the determination of cadmium level. In the laboratory, 1 g of the samples (liver and kidney) was weighed into the digestion flask. To each portion of the sample in the flask, 5 ml of perchloric acid and 15 ml of 0.1 N concentrated HNO_3 in a ratio of 1:3 were added and then heated in an electric plate until the sample became clear. After digestion, 5 ml of 20% HCl (0.1 N) was added to the content. The content of the flask was filtered using Whatman filter NO42 paper into a 100 ml volumetric flask and was made up to the mark with distilled water and then stored in a plastic reagent bottle, ready for Atomic Absorption Spectroscopy (AAS) analysis to determine the cadmium content.

2.6. Antioxidant And Lipid Peroxidase Assay

After centrifuging the blood sample at 3000 rpm for 5 minutes the serum was utilized for the antioxidant and lipid peroxidase assay.

2.7. Catalase Activity (Cat)

Distilled water, precisely 1 ml, was added to the blank test tubes, while 62.5 µl of the sample was accurately measured and added to the labeled test tubes. Subsequently, 625µl of hydrogen peroxide was added to the labeled and blank test tubes. After three minutes, both sets of tubes received 1ml of 0.01M potassium permanganate and 125 µl of 6M H_2SO_4. Absorbance readings were then taken at 480nm within a 30 to 60-second timeframe. To prepare the spectrophotometric standard, 1 ml of 0.01 M potassium permanganate was mixed with 1 ml of 0.05 M phosphate buffer (pH 7.0), along with 155 µl of sulfuric acid solution. A blank containing distilled water was used to calibrate the spectrophotometer.

2.8. Determination of Superoxide Dismutase (SOD)

The test method, originally proposed by (10) and subsequently modified by (11), serves as the foundation for this study. Test tubes were filled with 2.5 ml of carbonate buffer, followed by the addition of 0.2 ml of tissue (heart) homogenate and proper labeling. A reference test tube was prepared similarly but with 0.2 ml of distilled water. Each test tube, including the reference, received 0.3 ml of a newly prepared adrenaline solution at a concentration of 0.3 mM. The contents were thoroughly mixed, and the absorbance at 420 nm was measured using a UV-visible spectrophotometer every 30 to 120 seconds. The spectrophotometer was calibrated using distilled water.

2.9. Determination of Malondialdehyde (MDA)

The assessment of lipid peroxidation followed the method outlined by (12), which involved measuring the formation of thiobarbituric acid reactive compounds (TBARC). This process entailed combining 1ml of serum (diluted to 0.5 ml) with a solution comprising 0.375% w/v thiobarbituric acid, 15% w/v trichloroacetic acid (TAC), 0.25 M HCl, and 1:1 v/v TA-TBA. The solutions were then boiled in a water bath for 15 minutes, cooled, and subjected to centrifugation at 3000 rpm for 10 minutes. Absorbance at 535 nm was measured against a reference blank, and the concentration of malondialdehyde in the sample was calculated using an extinction coefficient of 15–10 M cm.

2.10. Determination of Glutathione (GSH)

One milliliter of 5% trichloroacetic acid (TCA) was dispensed into a plain bottle, followed by the addition of 250 µl of the sample. The mixture was allowed to stand for 5 minutes before centrifugation. After centrifugation, 25 µl of the supernatant was transferred to another plain bottle, and 250 µl of Ellman's reagent along with 1.5 ml of phosphate buffer was added to the supernatant. The same procedure was repeated for the remaining test groups. Glutathione (25 µl) served as the spectrophotometric standard. Absorbance readings were taken at 412 nm against the reference standard.

Note: To prepare Ellman's reagent, dilute 1g of sodium trisilicate in 100 ml of distilled water and add 0.4 g of DTMB.

2.11. Hematological Parameter Tests

Using a micropipette, 3.99 ml of Hayem's solution was drawn, and 0.1 ml of blood was added for erythrocyte counting. After thorough mixing, a droplet of the mixture was placed onto a graticule slip covering the counting zone, ensuring the coverslip was dry and clean. The pipette tip was positioned to touch the slide of the coverslip, and by gently depressing the stopper, a small amount of blood was drawn passively through capillary action and examined under a microscope. Automated methods were employed for leukocyte assessment, crucial for evaluating immune system functionality. Platelet counting was conducted using the QBC tube post-centrifugation and reading on the QBC reader. Meanwhile, packed cell volume was determined through centrifugation and analysis using a hematocrit reader.

2.12. Statistical analysis

The data were expressed as means + standard error of the mean. The significance of mean values of different parameters between the treatment groups and control group were analyzed using one-way analysis of variance (ANOVA) after ascertaining the homogeneity of variances between the groups. Turkeys multiple comparisons were done by calculating the significance at $P<0.05$. The analysis was carried out using Graph Pad Prism 6.0.

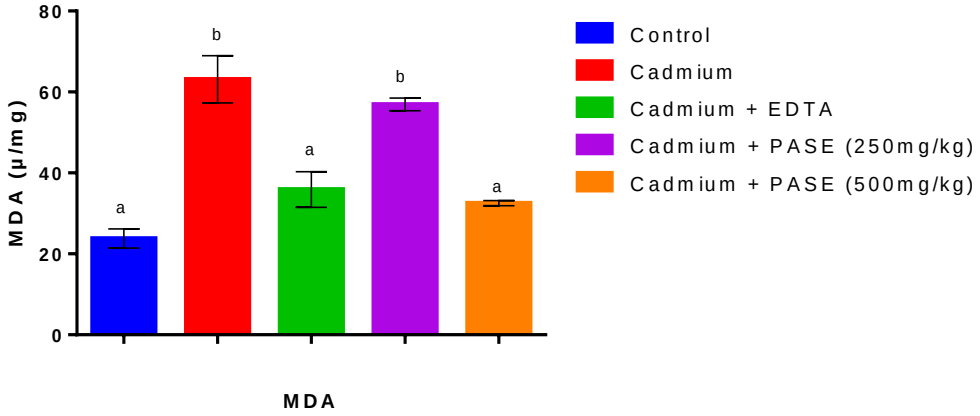
3.0. Results

Table 1: Effect of *Persea americana* seed extract on the antioxidant enzymes of wistar rats exposed to cadmium.

Sample	Mean ± Standard Error of Mean		
	SOD	**CAT**	**GPX**
Control	5.045 ± 0.235^{a}	212.7 ± 2.295^{a}	2.925 ± 0.015^{ab}
Cadmium (8 mg/kg)	3.150 ± 0.770^{a}	186.0 ± 4.030^{b}	1.625 ± 0.285^{b}
Cadmium (8 mg/kg) + EDTA	4.870 ± 1.060^{a}	215.9 ± 5.325^{a}	2.450 ± 0.440^{ab}
Cadmium(8 mg/kg) + PASE (250 mg/kg)	3.765 ± 0.075^{a}	192.4 ± 2.465^{b}	2.070 ± 0.070^{ab}
Cadmium (8 mg/kg) + PASE (500 mg/kg)	5.240 ± 0.590^{a}	231.7 ± 1.225^{a}	3.020 ± 0.080^{a}

Values represent mean ± standard error (n=6 per treatment). Dissimilar letters (a, b, c) are significantly different ($P<0.05$) from each other. PASE = *Persea Americana* seed extract, EDTA= Ethylenediamine tetra acetic acid.

The Table 1 shows the effect of *Persea americana* on the level of antioxidant (SOD, CAT, and GPx) of wistar rats exposed to cadmium. There was significant difference ($p<0.05$) in CAT level of the control group, EDTA group and PACE 500 mg/kg when compared to the group administered cadmium only and treatment group PACE 250 mg/kg. Statistically significant variations ($p<0.05$) in GPx levels were noted across the control group, the EDTA group, and the group PASE 250 mg/kg when compared to both the group given cadmium only and the PASE 500 mg/kg group. Additionally, a significant difference ($p<0.05$) was evident between the treatment groups administered PASE 250 mg/kg and PASE 500 mg/kg.



Values represent mean ±standard error (n=6 per treatment). Dissimilar letters (a, b, c) are significantly different (P<0.05) from each other. PASE = *Persea americana* seed extract, EDTA= Ethylenediaminetetra acetic acid.

Figure 1: Shows the Effect of *Persea americana* seed extract on Malondialdehyde (MDA) level of wistar rats exposed to cadmium**.**

Significant differences (p<0.05) were observed in the MDA levels among the control group, the treatment group receiving EDTA, and the group receiving PASE 500 mg/kg when compared to both the group given cadmium only and the group administered PASE 250 mg/kg.

Table 2: Effect of *Persea americana* seed extract on the haematological parameters of wistar rats exposed to cadmium

	Mean ± Standard Error of Mean							
	WBC	**LYM**	**MON**	**GRAN**	**RBC**	**HGB**	**HCT**	**PLT**
Control	6.30±1.80^{a}	1.00±0.20^{a}	0.45±0.25^{a}	0.30±0.20^{a}	4.90±0.60^{a} b	9.85±0.85^{a}	43.05±5.15^{a}	179.00 ±0.0^{ab}
Cadmium	7.85±2.25^{a}	6.95±2.25^{b}	1.10±0.80 a	1.30±0.70^{a}	7.60±0.10 a	12.65±0.95^{a}	32.30±13.00^{a}	695.0±162.0^{a}
Cadmium + EDTA	9.75±0.35^{a}	5.65±0.45^{ab}	0.85±0.05 a	0.35±0.05^{a}	6.40±0.60 ab	10.70±0.50^{a}	37.05±1.35 a	230.0±71.0 ab
Cadmium + 250mg/kg PASE	6.25±3.15^{a}	3.10±0.40^{ab}	0.75±0.45 a	0.25±0.15^{a}	3.95±0.55 b	7.85±2.25 a	38.65 ±1.55^{a}	95.50±6.50 b
Cadmium + 500mg/kg PASE	8.25±0.15^{a}	7.40±0.10^{b}	0.35±0.05 a	0.35±0.15^{a}	7.50±0.80 a	13.75±0.35^{a}	48.60 ±8.70^{a}	224.1±134.9^{ab}

Values represent mean ± standard error (n=6 per treatment). Different letters (a, b, c) show significant differences (P<0.05) between groups. WBC= White blood cell, LYM= Lymphocyte, MON= Monocyte, GRAN= Granulocyte, RBC= Red blood cell, HGB= Haemoglobin, HCT= Haematocrit, PLT= Platelet

Table 2 shows the effect of *Persea americana* seed extract on hematological parameters on cadmium-induced toxicity in wistar rats. In the case of lymphocytes, the control group exhibited a significant difference ($p<0.05$) compared to all other groups. Both the EDTA treatment group and the group administered PASE 250mg/kg demonstrated a significant difference ($p<0.05$) compared to the group given cadmium only and the group receiving PASE 500mg/kg. Concerning red blood cell count, both the control group and the EDTA treatment group exhibited a significant difference ($p<0.05$) compared to the group given cadmium only, as well as the groups administered PASE 250mg/kg and 500mg/kg. Moreover, the group receiving PASE 250mg/kg displayed a significant difference ($p<0.05$) compared to the group that was given cadmium only and the group that administered PASE 500mg/kg. For platelet count, the control group, the EDTA treatment group, and the group receiving PASE 500mg/kg demonstrated a significant difference ($p<0.05$) compared to the group given cadmium only and the group administered PASE 250mg/kg. Additionally, there was a significant difference ($p<0.05$) between the group given cadmium only and the 250mg/kg PASE group Values represent mean ± standard error (n=6). Dissimilar letters (a, b, c) are significantly different (P<0.05) from each other.

PASE= *Persea americana* seed extract, EDTA= Ethylenediamine tetra acetic acid

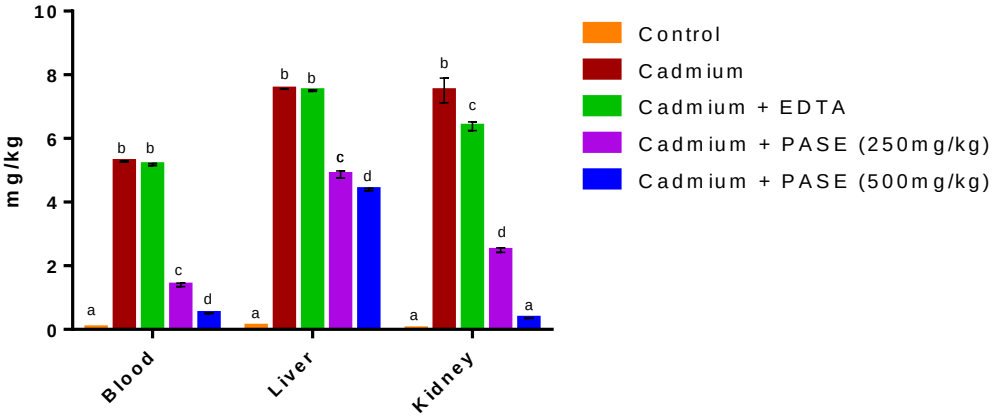


Figure 2: Shows Cadmium concentrations (mg/kg) of treatment groups in the blood, liver and kidney tissues.

Significant differences ($p<0.05$) in cadmium levels were observed in the blood and liver of Wistar rats in the control group compared to all other groups. The group given cadmium only and the EDTA treatment group exhibited significant differences ($p<0.05$) compared to the groups administered PASE 250mg/kg and 500mg/kg. Moreover, there was a significant difference ($p<0.05$) between the groups administered PASE 250mg/kg and 500mg/kg. In the kidney, significant differences ($p<0.05$) were noted across all groups when compared with each other.

3.1. Discussion

Cadmium, recognized for its significant toxicity as a heavy metal, presents risks even with minimal exposure, causing acute and chronic effects on both human health and the environment. Cadmium is transported in the bloodstream, predominantly bound to erythrocytes and significant protein complexes such as albumin, with a minor portion potentially binding to metallothionein. The liver and kidneys collectively store 50-75% of the body's total cadmium content (13). The purpose of this study was to evaluate the chelating effect of *persea americana* (avocado) seed on organ-tissue parameters of cadmium induced toxicity in wistar rats.

CdCl is acknowledged for its capacity to induce lipid peroxidation, yet its effect on antioxidant enzymes remains debatable. While some studies propose that Cd may instigate oxidative damage by promoting membrane lipid peroxidation through the inhibition of antioxidant enzymes [14], others suggest that Cd exposure could enhance antioxidant enzyme activities, thus mitigating lipid peroxidation [15]. Despite its incapacity to directly generate free radicals under physiological conditions, lipid peroxidation is regarded as the primary mechanism driving Cd toxicity [16]. This process triggers oxidative stress via the Fenton reaction, resulting in the production of hydroxyl radical species that initiate lipid peroxidation [17]. Breakdown products of lipid hydroperoxide, such as MDA and 4-hydroxynone-nal, can form disruptive cross-links with proteins and nucleic acids, contributing to carcinogenesis. Excessive generation of reactive oxygen species (ROS) can induce cellular damage when it exceeds the capacity of antioxidant defense systems, including enzymes like SOD, CAT, and reduced GSH [18, 19, 20]. A study conducted by [21] observed significantly higher lipid peroxidation (MDA) and reduced activities of CAT, GPx, and SOD in human erythrocytes with increasing concentrations of CdCl treatment. In this study, administration to cadmium group led to decreased levels of SOD, CAT, and GPx, as well as increased MDA levels compared to the control group. However, treatment with EDTA, PASE 250mg/kg, and 500mg/kg resulted in increased levels of SOD, CAT, and GPx, and decreased levels of MDA compared to the cadmium-only group. Specifically, significant improvements were observed in the EDTA and PASE 500mg/kg treatment groups. During exposure to cadmium stress, EDTA mitigated the buildup of Reactive Oxygen Species (ROS) by initiating the activation of antioxidant enzymes. The presence of carotenoids, minerals, phenolics, vitamins, fatty acids, saponins, tannins, flavonoids, glycosides, alkaloids, phenols, and steroids in avocado seeds contributes to their antioxidant activity [22, 23]. The antioxidant potential of *Persea americana* seed extract might be attributed to its phenolic compound content, as demonstrated in a study conducted by [24], These compounds, as shown by [25], exhibit the capability to neutralize free radicals, remove free oxygen, and bind catalytic metals. This indicates promising potential in alleviating oxidative stress, averting different diseases, enhancing overall health, and retarding the aging process.

In comparison to the control group, the cadmium-only group showed increased levels of various blood parameters (WBC, LYM, MON, GRAN, RBC, HCT, HGB, and PLT) with significant increases in RBC, PLT, and LYM, and a decrease in HCT. Treatment with EDTA and PASE 500 mg/kg led to increased WBC levels, while PASE 250 mg/kg resulted in a decrease. MON and GRAN levels decreased with treatment, while HCT increased. HGB decreased with EDTA and PASE 250 mg/kg, but increased with PASE 500 mg/kg. PLT levels significantly reduced with treatment, and LYM decreased with EDTA and PASE 250 mg/kg but increased with PASE 500 mg/kg. RBC levels decreased across treatments, with significant decreases in the EDTA and PASE 250 mg/kg groups.

White blood cells (WBCs) are pivotal in inflammation and immune responses. Comprising neutrophils, lymphocytes, monocytes, and granulocytes these immune cells are essential for the body's defense. Neutrophils, the predominant circulating WBCs, quickly move to inflammation sites, undertaking phagocytosis to clear microorganisms, releasing antimicrobial molecules, and aiding in the recruitment of other immune cells [26]. Cadmium, functioning as an immunotoxic inhibitor, directly engages with immune cells, modifying their condition and capabilities, thus undermining the immune system in ways contingent upon both duration and dosage [27]. [28] observed an elevation in total white blood cell count following the administration of cadmium. This is frequently interpreted as a defensive reaction by the immune system aimed at counteracting potential harm induced by cadmium toxicity. Bioactive components found in avocado seed extract, including phenolic compounds, vitamins, and fatty acids, have been noted for their antioxidant and anti-inflammatory attributes. When exposed to cadmium-induced oxidative stress and inflammation, these compounds potentially work together to modulate the immune system. This modulation could result in heightened lymphocyte levels, essential for adaptive immunity, while decreasing monocytes and granulocytes, which participate in inflammatory reactions. [29] conducted a study indicating that avocado seed extract possesses anti-inflammatory properties. [30] observed elevated levels of red blood cells (RBC), hemoglobin (HGB), and hematocrit (HCT) in newly hatched Gallus gallus domesticus chicks exposed to cadmium. Notably, morphometric analysis indicated a dose-dependent increase in both the length and width of erythrocytes, along with an increase in the length and width of the nucleus within these cells. This suggests significant morphological alterations in response to cadmium exposure, shedding light on its potential impact on erythrocyte structure and function. Yet, there were reservations about the research by [30] likely influenced by factors such as the choice of experimental animal, duration of administration, and dosage. The elevation in RBC and HGB levels, combined with a reduction in HCT observed in rats given cadmium, may result from cadmium's toxic impact on erythropoiesis. This could lead to an augmented production of erythrocytes, while hematocrit levels are compromised due to factors like hemolysis or impaired red blood cell maturation. The reduction in RBC and HGB levels, alongside an elevation in HCT seen in cadmium-exposed rats treated with avocado seed extract, might stem from the extract's protective properties against cadmium-induced erythropoietic toxicity. This could result in hematocrit levels returning to normal due to enhanced erythropoiesis and minimized hemolysis or damage to red blood cells caused by cadmium exposure. Platelet activation involves intricate cellular signaling pathways, engaging various substances like platelet agonists or ROS, acting as second messengers to stimulate the arachidonic acid metabolism and phospholipase C pathway. Excessive production of these compounds can induce heightened oxidative stress, which intricately regulates thrombosis components, including platelet activation, critically impacting cardiovascular diseases [31]. [32] illustrated an increase in platelet count in cadmium-exposed rats. Conversely, [33] observed that avocado peel and seed extracts inhibited agonist-stimulated platelet aggregation, suggesting a potential counteractive influence on platelet activity. The effects on platelet function may manifest through various mechanisms, such as structure-dependent interferences, inhibition of ROS production, reduction of oxidative burst, modulation of specific pathways, or blocking of agonistic substances [34].

In rats administered only cadmium, there were significant increases in blood, liver, and kidney cadmium levels compared to the control group. Heightened exposure to cadmium in the bloodstream is associated with elevated blood pressure and hypertension. The findings indicated an increase in cadmium levels in both the liver and kidney following exposure, aligning with [35] research, which affirmed cadmium-induced hepatic and renal injury in rats exposed over time,

likely implicating hepatic cadmium metallothionein in nephrotoxicity. Within the liver, the cysteine-metallothionein complex contributes to hepatotoxicity before traveling to the kidney and accumulating in renal tissue, precipitating nephrotoxic effects. Cadmium has an affinity for binding with cysteine, glutamate, histidine, and aspartate ligands, potentially leading to iron deficiency [36]. Given that cadmium and zinc share similar oxidation states, cadmium can displace zinc in metallothionein, hampering its role as a cellular free radical scavenger. In the kidney, the body's limited ability to excrete cadmium exacerbates its adverse effects, with cadmium predominantly reaching the kidney in the form of cadmium-metallothionein (Cd-MT). Cadmium is reabsorbed in the proximal tubules of the kidney, hindering its excretion and fostering long-term accumulation, particularly within the kidney, which culminates in tubular cell necrosis [37]. Treatment with EDTA and PASE 250 mg/kg and 500mg/kg led to reductions in blood and liver cadmium levels, particularly significant in the PASE 250 mg/kg and 500mg/kg groups. Similarly, all treatment groups showed significant reductions in kidney cadmium levels compared to the cadmium-only group. According to studies, EDTA is known to be a chelating agent for heavy metal toxicity.

Studies suggest that avocado seeds contain a diverse array of beneficial compounds, including saponins, alkaloids, flavonoids, polyphenols, quercetin, and alcohol glucose, as well as essential amino acids like tryptophan and lysine, calcium, iron, tannins, sulfur, and vitamins A, B, and C. These components are recognized for their ability to enhance the kidney's glomerular filtration rate, facilitating the excretion of cadmium through urine. Additionally, the flavonoids found in avocado seeds disrupt the production of Reactive Oxygen Species (ROS), thereby inhibiting urolithiasis and guarding against oxidative stress-induced cell damage. Avocado pear seeds are abundant in carotenoids, minerals, phenolics, vitamins, fatty acids, saponins, tannins, flavonoids, glycosides, alkaloids, phenols, and steroids, which contribute to their antioxidant properties and aid in preventing liver damage. Moreover, flavonoids and polyphenols increase lipase lipoprotein activity, mitigating hyperlipidemia and related conditions such as liver damage. The soluble fiber in avocado seeds assists in reducing cholesterol levels by binding to cholesterol and eliminating it from circulation. Past studies have demonstrated the anti-hypertensive effects of avocado seeds, traditionally used in Nigeria to address high blood pressure, attributed to their potassium content, which helps regulate healthy blood pressure levels by balancing sodium levels. Furthermore, significant variations between doses indicate that the efficacy of avocado seed extract is dependent on dosage. Consequently, *Persea americana* (avocado) seeds exhibit chelating effects on cadmium in the blood, kidney, and liver.

4.0. Conclusion

This study has practically proven that *persea americana* seed extract have a potential chelating effect on cadmium in the blood, kidney, and liver of wistar rats. It was also observed that the effectiveness of the extract is dose-dependent. Therefore, the dosage of the extract must be taken into consideration when administering. Further studies are needed to evaluate the safety of avocado seed for long-term administration and understanding of its mechanistic effect.

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