EVALUATION OF AVOCADO PEEL EXTRACT AS A POTENTIAL AGENT FOR AMELIORATING CARDIOTOXICITY INDUCED BY LEAD AND CADMIUM CO-ADMININSTRATION IN RAT

BY

Emmanuella Oghogho CLEMENT (Miss)

LSC1706016

UNIVERSITY OF BENIN

BENIN CITY

SEPTEMBER, 2023.

EVALUATION OF AVOCADO PEEL EXTRACT AS A POTENTIAL AGENT FOR AMELIORATING CARDIOTOXICITY INDUCED BY LEAD AND CADMIUM CO-ADMININSTRATION IN RAT

BY

Emmanuella Oghogho CLEMENT (Miss)

LSC1706016

A THESIS WRITTEN IN THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY AND SUBMITED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELORS (B.Sc.) DEGREE IN THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

SEPTEMBER, 2023.

CERTIFICATION

This is to certify that the project was carried out by Emmanuella Oghogho CLEMENT (Miss), Matriculation Number LSC1706006, a 500 Level student of the department of Science Laboratory Technology, Faculty of Life sciences, University of Benin, in partial fulfillment of the requirement for the award of a Bachelor of Science degree, B.Sc. (Hons.) in Science Laboratory Technology.

Mr. E. Osazuwa **(Project Supervisor)**	**Date**
Dr. Osumah **(Project Coordinator)**	**Date**
Prof. E.O. Oshomoh **(Head of Department)**	**Date**
External Examiner	**Date**

DEDICATION

This work is dedicated to God Almighty for His guidance, mercy, love and direction given to me to complete this project work and also to my parents, for their constant support and care.

ACKNOWLEDGMENTS

I thank God almighty who is my redeemer and Author of knowledge for the special grace to complete my project and also write this report .I am grateful to my parents Mr. and Mrs. AYOMIKE for their support and guidance and much gratitude to my supervisor Mr. Ekhator Osazuwa who took his time to ensure and see that my project is properly attended to and also to those that works with them.

Special thanks to my lovely grandmother Mrs. Veronica Omoruyi and my aunties and uncles Josephine Omoruyi, Mabel Omoruyi, Edowaye Omoruyi, Benjamin Omoruyi, Samuel Omoruyi for their financial support and encouragement.

And to my wonderful siblings Courage, Hassan, Damilare, Feranmi, Osayi, David, Felix, Ruth and Divine. To my friends Anu, Titilayo, Ose, Osaymen and Hauwa thank you all for support.

TABLE OF CONTENT

LIST OF TABLES

LIST OF Figures

Abstract

Recycling fruit peel trash has not only helped to reduce solid waste concerns, but it has also assisted in the discovery of key compounds that have been found to have significant uses. Fruit peel wastes may contain useful components similar to those found in fruit. These beneficial compounds can be employed to create pharmaceutical/medicinal, nutritional, and energy-rich formulations. The study seek to evaluate of Avocado peel extract as a potential agent for ameliorating cardiotoxicity induced by lead and cadmium. The fresh avocado fruits were obtained from a local market (Oba market Oredo) Local Government of Edo state. The seed was extracted, cleaned and prepared. The following test was carried out lipid profile and antioxidant and lipid peroxidation. The level of heavy metal present in the heart showed least in lead and cadmium concentration in group four and five compared the other groups. In the administration of the extract showed increase in uric acid, HDL, LDL, direct bilirubin and total bilirubin in the heart on male albino rat when compared to control. From the result, the administration of the extract showed decrease in triglyceride level in all doses when compared to control except for the avocado peel extract with a 100 mg/ml showed increase when compared to control. From the result, the administration of the extract showed increase at significant ($p < 0.05$) in Creatinine and Albumin level on male albino rat when compared to control. The administration of the extract showed increase at significant ($p < 0.05$) in MDA, SOD and CAT level in the heart on male albino rat when compared to control. The administration of the avocado peel extract with doses of 75 mg/ml and 100 mg/ml showed significant decrease in MDA, SOD levels in the blood on male albino rat when compared to control and also, the avocado extract at dosage of 75 mg/ml showed significant increase in CAT levels in the blood on male albino rat when compared to control. While at dosage of 100 mg/ml showed decrease at significant ($p < 0.05$) in CAT levels in the blood on male albino rat when compared to control. The avocado peel extract showed significant decrease in MDA in the testes on male albino rat when compared to control. The ethanol avocado peel extract showed significant increase in, SOD, and CAT levels in the testes on male albino rat. The protective effect of avocado peel against cadmium and lead toxicity, owing to its polyphenol and flavonoid content represents a potential avenue for treating heavy metal-related health conditions.

CHAPTER ONE

INTRODUCTION

Traditional uses of plants in ethnomedicine encompass the diagnosis, treatment, especially therapy of many afflictions, illnesses, as well as poisoning, where they may heal or decrease toxicity (Shil *et al*., 2014). Many plant items have exterior skins that are commonly peeled off and thrown away since they are typically unusable and unpalatable. Hard seeds and peels that were previously inedible are typically identified as phytowastes along with disposed of as such. Street as well as market fruit sellers, as well as businesses that process fruit, produce a lot of garbage, which, unless managed and utilized properly, may be unhealthy for the natural world and induce sickness. Repurposing them as a substitute supply of antioxidant may therefore lead to low-cost innovative generational remedies, quantifiable economic gains for the pharmaceutical as well as nutraceutical businesses, and a decrease in contamination (Duda-Chodak and Tarko, 2007).

Fruit peel scraps may include beneficial elements that are comparable to those within fruit. Pharmaceutical/medical, nutritional, as well as full of energy compositions may be made using these advantageous substances (Shil *et al*., 2014). Recovering fruit peel waste has not merely assisted with solid waste issues, yet it has additionally assisted in the identification of important chemicals that have been shown to have important applications. Aside from appropriate distillation, commercial extraction, technological integrating, and fruit peel administration, phytochemicals of interest may additionally be recovered.

In order to isolate and extract secondary compounds, fruit peel extracts have become one of the main sources (Altemimi *et al*., 2017). In addition to their therapeutic medicinal effects on a

variety of illnesses like osteoarthritis, cardiovascular conditions, diabetes, as well as cancer, that have been scientifically demonstrated and recorded through numerous in vitro, in vivo, alongside clinical stage trials throughout various cultures as well as societies. These include mangiferin, gallic acid, flavonoids, catechin, as well as gallic acid derivatives, which are which are highly rich in bioactive elements (Shil *et al*., 2014).

1.1 Avaocodo peel

In addition to having a significant number of bioactive components, avocado peel also includes carbs, proteins, lipids, as well as fiber (Colombo and Papetti, 2019). According to reports, the peel has a significant level of phenolic compounds and antioxidant properties. Additionally, it has been shown that the peel possesses potent antibacterial, antibiotic, as well as anti-inflammatory activities (Sadiye, 2021). It is a potential substance within this context for the creation of medicinal & nutritional food items, as well as it may also be employed as a bio-source towards the creation of environmentally friendly adsorbents (Sadiye, 2021).

When opposed with different fruit items, avocado peels have a higher concentration of phytochemicals, which are significant additional metabolic components generated by plants (Sadiye, 2021).

Avocado peel has reportedly been shown to be rich in phytochemicals, including polyphenol. According to Ahangarpour *et al*. (2019), phenolic phytochemicals may be related to a variety of health advantages, including anticancer, anti-aging, antioxidant, as well as anti-inflammatory activities. They are widely utilized in the food sector because of their anti-oxidant and color-preserving properties, as well as their ability to stop the growth of germs and molds (Kosiska *et al*., 2012).

1.2 Lead

Lead is a particularly important heavy danger substance within the natural environment. Due to its important physico-chemical properties, its application could date back to the beginning of time. On a global scale, it is a widespread, major, and dangerous ecological substance (Wani et al., 2015). Due to its important characteristics, such as brittleness, flexibility, ductility, poor conductivity, and resistance to corrosion, it is difficult to completely eliminate it. Due to continued use as well as non-biodegradable construction, the quantity discovered within the environment grows along with the associated concerns. The exposure of humans towards lead as well as their compounds primarily happens in lead-associated occupations with a a range of sources such as performed gasoline, industrial operations such as smelting of lead as well as burning, pottery, boat construction, lead that included pipes, battery reuse and recycling, grids, the arm industry, pigments, as well as book production (Wani *et al*., 2015).

1.3 Cadmium

Humans are mostly exposed to Cd through eating or breathing. According to the particle size, cadmium dust breathed is taken in 10 to 50 percent of the time. There is very little absorption via skin contact. According to the size of the particle, only 5–10% of swallowed Cd is absorbed. People who are calcium, or zinc deficient have higher gastrointestinal absorption (Bernhoft, 2013). The primary source of human contamination with cadmium is thought to be smoking tobacco (Bernhoft, 2013). Smokers routinely have greater blood as well as kidney concentrations of Cd than non-smokers. In work environments, inhalation brought on by workplace exposure can prove considerable. A significant chemical pneumonitis, for instance, might be caused by

welding or soldering (Bernhoft, 2013). Either industrial or organic sources, heavy metals that resemble other toxic compounds can seriously endanger human life (Cobb, 2008). The chemical element cadmium (Cd), which has the atomic numbers 48 as well as 112, as well as the melting as well as boiling points of 321 as well as 765 degrees Celsius correspondingly, is soft, malleable, silvery white with a blue tint, glossy, as well as electropositive.

1.4 Statement of the Problem

Contamination from the environment like lead and cadmium can reach the body via a variety of channels, such polluted food, water, as well as air. Cardiotoxicity, which includes oxidative stress, inflammation, as well as structural harm to heart tissue, is believed to be associated to chronic exposure to certain heavy metals. There are currently few efficient treatment therapies available to stop or lessen the cardiotoxic consequences of heavy metal exposure. The efficiency of conventional therapies is frequently constrained, and they may also have unfavorable side effects. With its bioactive components, such as as polyphenols as well as antioxidants, that have shown protective properties versus oxidative stress as well as inflammation, avocado (Persea americana) peel preparation has drawn interest. Its ability to safeguard against the cardiotoxicity caused by heavy metals is still untapped. Preclinical models must be thoroughly evaluated prior to avocado peel extract may be regarded as a promising cardioprotective drug. Rats are useful models for analyzing the possible defensive properties of natural substances because of their physiological as well as genetic similarity to humans. In order to assess if avocado peel extract may mitigate the cardiovascular risks of lead as well as cadmium co-administration within rats, it is necessary to research these consequences in more detail.

1.5 Aim and Objectives

Evaluation of Avocado peel extract as a potential agent for ameliorating cardiotoxicity induced by lead and cadmium co-admininstration in rat.

Objectives of the Study:

- To determine the effect of avocado peel extract on hermatological parameters of heavy metals (lead and cadmium) on the heart
- To determine the effect of avocado peel extract on antioxidant status (GPS,SOD,MDA)
- To determine the effect of avocado peel extract on the histology of the heart
- To determine the effect of avocado peel extract on the level of lead and cadmium.

1.6 Significance of the Study

The study explores the potential use of avocado peel extract, a natural product rich in bioactive compounds, as a cardioprotective agent. If successful, this investigation could lead to the development of cost-effective, safe, and natural interventions to counteract the harmful effects of heavy metals on the heart, offering an alternative to conventional treatments with potential side effects.Preclinical studies in animal models, such as rats, are essential steps in assessing the safety and efficacy of potential therapeutic agents. The findings of this study could pave the way for further research, including clinical trials, and may eventually lead to the development of avocado-based cardioprotective supplements or pharmaceuticals. The study's outcomes have the potential to inform public health policies and recommendations related to heavy metal exposure reduction and cardiovascular health. If avocado peel extract proves effective in mitigating cardiotoxicity, it could have implications for dietary recommendations and preventive strategies

for individuals at risk of heavy metal exposure. The significance of this study lies in its potential to shed light on a critical health issue, explore natural remedies, offer insights into preventive strategies, and contribute to the broader fields of environmental health and sustainable agriculture. Ultimately, the findings of this research may have far-reaching implications for improving human health and mitigating the negative impart of heavy metal contamination on cardiovascular well-being.

CHAPTER TWO

LITERATURE REVIEW

Avocado is a type of dicotyledonous plant that is a member of the Ranales order and family of Lauraceae. Gaertner and Miller categorized it as Persea gratissima as well as Persea americana, respectively. Geographic isolation caused P. americana to diversify into subspecies, which eventually gave rise to several botanical kinds (Yahia and Woolf, 2011). Among the few species of the genus Persea with major economic value is the avocado tree.

Persea americana, sometimes stated to as the avocado or avocado pear, are a flowering plant belonging to the Lauraceae family that is native throughout Mexico as well as Central America (Bhuyan *et al*., 2019). According to Cowan *et al*. (2016), the avocado fruit constitutes a berry having a single sizable seed. The world's largest grower of avocados is Mexico (Bhuyan *et al*., 2019). According to Taulavuori *et al*. (2013), "superfood" means foods which are advantageous to human beings because they include high concentrations of nutrients as well as phytochemicals with biological activity like antioxidants. Avocado in particular has lately experienced a sharp increase in demand (Rahmani *et al*., 2017) as well as is frequently referred to as a "superfood" due to its distinct nutritional as well as phytochemical makeup in comparison with various other fruits.

2.1 Vehicular Name

According to Bhuyan *et al*. (2019), it is normally mentioned to as avocado pear or avocado throughout the United States. According to Nwauzoma and Dappa (2013), it is referred to as "Igba/apoka" within Yorubas in the southern area of Nigeria as well as "Ube-beke" amongst Igbo.

2.2 History and Origin of the Avocado Plant

The avocado is a fruit that has an estimated 10,000-year history that is grown on tropical trees as well as has a pear-shaped, blackish-green color, a creamy texture, and a distinct flavor (Birnbaum *et al*., 2003; Cervantes-Paz, and Yahia, 2021). It also has a high amount of nutrients. Avocado is referred to as "Green Gold" and has a high value owing to its significance in the marketplace. The earliest Avocado remains were discovered in Coaxcatlan, Mexico, some 10,000 years ago (Ayala and Ledesma, 2014; Galindo-Tovar *et al*., 2007). The Aztecs, Olmecs, as well as Maya were three ancient civilizations in North, Central, as well as South America who revered avocados as a gift from God (Ayala and Ledesma, 2014). The Tehuacan Valley throughout Mexico may have been home to initial human communities as far back as 8000–7000 BC, while the Mesoamerican group could have cultivated the seeds around 5000 BC (Galindo-Tovar *et al*., 2007).

2.3 Distribution

Another of the earliest blooming plants in history recorded comprises the avocado (Persea americana Mill), which is a member of the genus Persea as well as family Lauraceae (Popenoe and Zentmyer, 1963). Every of the 81 species of Persea that exist today have their roots in the Old World, with the exception of the Persea indica species, that is native to the Canary Islands, Madeira, as well as the Azores. Southeast Asia is the place of origin of certain species (Popenoe and Zentmyer, 1963). According to Galindo-Tovar *et al*. (2007), Persea is split into the First

subgenus Eriodaphne, a group of plants that are resistant to Avocado root rot but regrettably have little commercial value because to the extremely low proportion of delectable meat. Avocado (Persea americana Mill) is a member of the second subgenus of Persea. The center was in northern Mexico, and it extended north as well as south, first to the southern United States and then to Brazil, Colombia, Bolivia, and Chile in the south. According to RMRDC (2012), the Southern as well as Central regions of Nigeria are the main distribution areas for avocado. The vegetation circumstances have a strong influence on the distribution's abundance, with the rainforest possessing a particularly prolific spread. There are four stages of avocado accessibility in Nigeria, each based on the fruit's quantity, economic/marketing possibility, degree of output at the time, and supply-chain stability. For States in which it accounts for more than 10% of the amount of non-wood forest species marketed as well as traded for economic purposes, avocado may be deemed plentiful, according to RMRDC (2012). Additionally, it displays the states where avocados account for 10% of the native vegetation and are widely produced on farms alongside private gardens. Yet, it is regarded as less plentiful in States wherein avocados make up between 6.0 and 9.9% of the native flora. While stands can be observed on farms, gardens, as well as as a component of wayside plantings, avocado planting has never been widespread in these States. RMRDC (2012) evaluated avocado abundance to be common in states where it makes up 3.0 through 5.9 percent of the plant life in the wild. While the plant is occasionally seen in farmsteads as well as backyard gardens, growing avocados in backyard farms as well as gardens cannot be extremely prevalent in these States. In states wherever avocados make up just about three percent of the native flora or in areas where the plant species is almost nonexistent, it is regarded as uncommon.

2.4 Occurrence

It's unclear when and how avocados were introduced to Nigeria. Yet, Nigerians were aware of avocados as early as the 1920s (RMRDC, 2012). Although it has existed there for a while, little to no attempt has been done to investigate its roots and the local variants that are accessible. However, the material that was available suggested that South American indigenous people had brought the fruit as far as Peru, as well as the Spanish had furthered its expansion throughout their colonies within Chile, West Africa, Madeira, as well as the Canaries (Hamilton, 1987). Through these locations, avocados were introduced to all areas with suitable soil as well as climate. Avocado types have recently migrated from California in the United States to Australia, New Zealand, as well as South Africa (Hamilton and Evans, 1999). In Nigeria, you may find avocado in the country's farms, residences, and forests. In addition to this, avocados are also cultivated in the Central region of the nation to a certain degree (RMRDC, 2012).

2.5 Description

The avocado plant stands upright and may reach heights of 9 towards 18 m, with a bole diameter of thirty towards sixty centimeters. The leaf may be alternating, dark-green, glossy on the top surface, as well as pale on the underside. The leaf may be lanceolate, oval, or obovate (Schaffer *et al*., 2013). According to Morton (1987), who examined the leaf dimensions, which ranged from 7.5 towards forty centimeters, the fruit is pear-shaped, frequently necked, oval or almost spherical, as well as is 7.5 to thirty-three centimeters in height as well as can be as broad as 15 cm. The fruit skin can be reddish-purple, nearly black in color. According to Abraham *et al*. (2018), the fruit can be smooth or pebbled, shiny or dull, slender or leathery with a skin thickness of up to 6 mm, flexible or granular, as well as brittle. It can also be speckled with small yellow spots. Although avocados often have all-white to rich-yellow flesh, some varieties have a small layer of tender, bright-green flesh directly behind the peel. The avocado fruit is oblate, conical,

or ovoid within shape, firm as well as heavy, ivory within color, and 5 to 6.4 centimeters long. Its solitary seed is encased in two brown, slender and papery seed coverings that frequently adhere to the flesh cavity.

Figure 2.1: Pictorial representation of *Persea Americana* tree

Figure 2.2: Pictorial representation of the fruit of *Persea americana*

2.6 Botanical Classification: *Persea americana M.*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Laurales

Family: Lauraceae

Genus: Persea

Species: P. Americana

Source: (Yasir *et al*., 2010)

2.7 Nutritional Composition

Avocados are widely valued for their rich nutritious content and medicinal uses. According to Ogunwusi and Ibrahim, (2016), the dietary content of avocado is displayed in Table 1. According to its size and variation, an avocado is said to have 585 towards 1000 kJ of energy throughout its full state. The main elements that significantly affect the nutrient content of avocados include variation, ripening grade, the weather, the makeup of the soil, as well as fertilizers (Duester 2000).

Table. 2.1: Nutritional Composition (on a fresh weight basis) of the Edible Part of Avocado Fruit

Component	Value (100g Flesh)	Method
Water (%)	74.9	Air oven
Fat (%)	19.8	Soxhlet Extraction
Protein (%)	1.9	Kjeldhal
Fibre (%)	1.7	Tricholoroacetic acid
Ash (%)	1.2	Tricholoroacetic acid
Sugars (%)		
Glucose	0.2	Differential
Fructose	0.1	Differential
Sucrose	0.1	Differential
Starch	0.1	Differential
Energy (Kj 100g 1)	914	Differential
Vitamins (mg %)		
Ascorbic acid	11.3	DCPIP
Thiamin	+	Alkaline oxidation
Riboflavin	++	Chromatography
Pamtothenic	++	Chromatography
Nicotinic acid	+++	Cynigen bromide colour reaction
Vitamin B6	++	Chromatography
Folic acid	+	Chromatography
Biotin	+	Chromatography
Carotenoids (mg %)	0.18	Chromatography
Minerals (mg %)		
Potassium	46.7	FES
Sodium	2.0	FES
Phosphorus	28.3	AAS
Calcium	22.3	AAS
Magnesium	22.3	AAS
Iron	0.6	AAS
Zinc	0.5	AAS

Adapted from (Ogunwusi and Ibrahim, 2016)

Table 2.2. Phytochemical analysis of *P. americana* seeds

Phytochemicals	**(mg/100g)**
Flavonoids	20.33±0.01
Tannins	0.76±017
Saponins	0.52±0.42
Oxalates	4.40±0.30
Phytates	0.44±0.01
Alkaloids	5.40±0.00

***Source*: (**Setyawan *et al*., 2021**)**

2.8 Pharmacological Effect

2.8.1 Vasorelaxant Activity

For the aorta of the rats rings having interaction endothelium precontracted having noradrenaline, the aqueous extract of P. americana (0.01 to 12.8 mg/mL) induced a dosage-related vasorelaxation reaction, having an EC50 of 0.88 mg/mL. The aqueous extract of P. americana considerably reduced the vasorelaxant activity within the endothelium-denuded rings. Rat aortic rings that had been precontracted using noradrenaline relaxed after receiving cumulative additions of acetylcholine, L-NAME, methylene blue as well as indomethacin (10-5 M), however, had no discernible influence on the vasorelaxant action. Noradrenaline as well as potassium chloride (10-80 mM) dosage-response curves were shifted to the right by the extract of avocado extract using aqueous as solvent with a dosage of one or five mg per mL. L-NAME or methylene blue actually prevented this action, indicating that the production as well as discharge of endothelium-derived relaxing factors are necessary for vasorelaxation. The

inhibition by indomethacin raises the possibility that avocado also functions by triggering PGI2 as well as PGE2 receptors. The suppression of Ca2+ mobilization via channels that is dependent on voltage as well as to a smaller extent, via receptor-operated valves might have a vasorelaxant impact (Owolabi *et al*., 2005).

2.8.2 Analgesic and Anti-Inflammatory Activity

The control writhes were significantly and dose-dependently inhibited by the aqueous extract of avocado leaves. 1600 mg/kg of extract had an inhibitory effect comparable to one hundred mg per kg of acid from acetylsalicylic (57.2 percent as well as 58.0 percent, correspondingly). The eight hundred mg per kg of the extract demonstrated the same level of inhibition (87.2 percent) as morphine with a dosage of two mg/kg and 87.0 percent. The extract significantly and dose-dependently inhibited both phases. In phase II of the test, the extract (800 mg/kg) inhibited more effectively (77.1 percent) than acetylsalicylic acid (68 percent) did. At 3 hours, the extract of avocado leaf extracted using aqueous as solvent with as dosage of eight hundred mg per kg significantly reduced the edema brought on by carrageenan. This impact was comparable to what indomethacin generated for the same amount of time (Adeboye *et al*., 2002).

2.8.3 Hypotensive Activity

The greatest examination dosage for i.p. injections turned out with a dosage of fifty milligrams per kilogram when avocado leaf aqueous as well as methanol extract was administered intravenously to normotensive anesthetized rats, which resulted in dose-dependent hypotensive reactions. A pilot experiment showed that doses beyond 50 mg/kg brought about death through rats inside ten minutes of administration. The impacts on hypotension were significantly distinct

in comparison to those for the control at doses higher than 12.5 mg/kg. Additionally, it showed that the amount of time of activity was dosage dependant (Adeboye et al., 1999).

2.8.4 Anticonvulsant Effect

Mice can experience an anticonvulsant response from aqueous avocado leaf extract. The plant extract may be capable of treating both petit mal as well as grand mal epilepsy as it performed so well in the experimental seizures paradigm. In comparison to bicuculline (BCL)-induced seizures, pentylenetetrazole (PTZ) as well as picrotoxin (PCT)-induced convulsions were more controlled by the plant's leaf extract. Convulsions often began later on average and lasted shorter on average, accordingly. These findings point to the possibility that avocado leaf aqueous extract might have improved, or perhaps in some ways interfered with, GABAergic activity &/or neurotransmission, hence inhibiting and/or attenuating PTZ-, as well as BCL-induced convulsions within the mice utilized (Ojewole & Amabeoku, 2006).

2.8.5 Antiviral Activity

In vitro study of replication of hindering impacts of the avocado dry leaf infusion as well as ethanol extract have been compared. The adenovirus type 3 (AD3), as well as ADV were the viruses selected for the first test. Only HSV-1 as well as ADV were evaluated using the ethanol extract. In contrast to the ethanol extract, which had no action in the experiments, the infusate was effective against all three viruses (De Almeida *et al*., 1998).

2.8.6 Wound healing Activity

Among the excision wound model, complete epithelialization (total healing) has shown in the animals on day 14 on average after oral or applied topically of avocado extract from fruit with a dosage of three hundred mg/kg/day. On the contrary hand, it took within 17 days for the

tracking equipment to fully recover. It was shown that comparison to the controls, the extract-treated wounds epithelialized quicker. The weight of granulation as well as hydroxyproline amount of the tissue obtained from the extract-treated animals utilized during the dead zone wound model were significantly higher than those of the controls (Nayak *et al*., 2008).

2.8.7 Antiulcer Effect

The purpose of the research was to find out whether P. americana aqueous leaf extract had any antiulcer properties. After giving groups of albino rats the ulcerogenic medications ethanol as well as indomethacin, the plant's aqueous leaf extract was given directly to these rats as a pretreatment. Indomethacin as well as ethanol-induced ulcers in rats were substantially and dose-dependently treated by the extract (Ukwe and Nwafor, 2004).

2.8.8 Antihepatotoxic Activity

Another team of researchers showed how avocados affect the liver. In a research on the antihepatotoxic activity in rats, Martins Ekor *et al*. P. americana's methanolic extract may offer defense from the toxicity as well as oxidative stress brought on by acute paracetamol intoxication. CAT, SOD, as well as glutathione peroxidase, resulting in are the main intracellular protection process designed to deal with increasing oxidative stress, are likely responsible for this protection's antioxidant effect. The avocado plant leaves extracted from methanol, didn't suggestively change the functions of the antioxidant enzymes determined (SOD as well as CAT) within normal rats; nevertheless, the extract substantially boosted the level of activity of these enzymes throughout damage to the liver brought on by acute paracetamol toxicity. A promising treatment for liver illnesses along with other pathologies linked to oxidative stress includes P.

americana's methanolic leaf extract as well as hepatoprotective effect versus acute paracetamol poisoning.

2.8.9 Antioxidant Activity

Persenone A along with B, which have distinct antioxidant activities, were discovered as well as identified by Kim *et al*. (2002) in avocado fruit. It's critical to recognize the components of this plant's leaf extract that have antioxidant qualities. The avocado plant leaves extracted from methanol has antioxidant properties, which protects the liver from acute toxicity from paracetamol, making it a possible treatment for liver disorders as well as serveral oxidative stress-related illnesses.

2.8.10 Hypoglycemic Activity

In normal rats, P. americana aqueous leaf extract has a diabetic impact. Following a single dosage of the extract, the greatest antidiabetic action was attained at 6 hours, resulting in a 60 % decrease within serum glucose levels (Anita *et al*., 2005).

2.8.11 Effect on body weight

In a prior study, it was shown which administering avocado plant leaves extracted from aqueous as well as methanol forms caused a reduction in body weight when contrasted with hyperlipidemic controls. The breakdown of lipids stored in adipose tissue may be accelerated by P. americana leaf extracts, which might lead to a reduction in average body weight (Litz *et al*., 2005; Pliego-Alfaro and Litz, 2007).

2.9 Hematological Parameter Test

2.9.1 Serum Lipid Profile

For the purpose of predicting cardiovascular risk, serum lipid profiles are already nearly a standard test (Nigam, 2011). The test has four fundamental components: total, HDL, LDL, and triglycerides. Usually, a sample of fasted blood is utilized. When we refer to fasting, we imply a 12- toward 14 hour period of complete nutritional restriction, with the exception of water as well as medicine. Due to two key factors, this may be accurate: (1) For several hours after eating, postprandial triglycerides are high (Campose *et al*., 2005): (2) Fasting blood samples are used to create the majority of reference values for serum lipids (NCEP, 2002) as well as European guidelines (De Backer *et al*., 2004) further advise performing a lipid profile on a fasting blood sample to determine the likelihood of cardiovascular disease (Nigam, 2011). The aforementioned suggestions do permit total as well as HDL inside the non-fasting specimen, though, as these lipids do not change significantly between fasting as well as non-fasting specimens. Furthermore, non-HDL (overall HDL) a secondary therapeutic agent within adult treatment panel III, could be applied in the non-fasting condition (NCEP, 2002; Nigam, 2011).

2.9.2 Troponin

Among the most important jobs assigned to the emergency physician is the diagnosis of cardiac crises. To provide patients with the necessary life-saving medications, the wide range of possible causes of chest discomfort must be swiftly and precisely narrowed down (Aakre *et al*., 2019). Numerous crucial diagnostic methods are utilized in conjunction with the history as well as a physical checkup to distinguish between the various causes of chest discomfort (Sharp *et al*., 2019). The estimation of troponins is one instrument that has developed into a crucial part of cardiovascular workups as well as diagnostics (Mirkin *et al*., 2019). During 1995, whenever the initial cardiac troponin T (cTnT) test was authorized, the cardiac troponins are currently utilized in clinical settings (Xu *et al*., 2013). After then, it has been evident that better cardiac specificity

as well as, in specific, increased sensitivity, particularly through the use of high-sensitivity troponins from the heart tests, contribute to a greater common and reliable diagnosis of cardiovascular problems (Potocki *et al*., 2012).

The three different molecular forms of troponin correlate to the unique isotypes of fast-twitch, slow-twitch, as well as cardiac tissue, respectively (Rasmussen and Jin, 2021). The amount of amino acid heterogeneity of the skeletal isotypes is around forty percent, which means they share a molecular dimension of 20,000 Daltons (Brotto *et al*., 2006). Along with having an extra 31 residues located at the amino terminus, the cardiac isotype differs from the skeletal isotypes in terms of sequence heterogeneity by roughly forty percent. As a result, cardiac troponin and skeletal troponin may be distinguished immunologically (Wei and Jin, 2016).

Troponins are located in the myocyte as structural (bound) proteins as well as a tiny free pool which is present within the cytosol; this free pool has a concentration of 3.5% for cTnI as well as a range of six to eight percent for cTnT (Garg *et al*., 2017). Immunological differentiation between cTnT as well as cTnI is possible due to differences in amino acid sequence (Wu and Feng, 1998). In contrast to skeletal forms, complexed ternary as well as binary types (cTnICT as well as cTnIC) as well as cytosolic cTnI or cTnT are unable to cross-react alongside cardiovascular types of either troponin (Potter *et al*., 2022). Before the discovery of troponin, myocardial damage was detected using a variety of biological indicators (Jacob and Khan, 2018). Aspartate transaminase, lactate dehydrogenase, as well as creatinine kinase, which were utilized in the 1960s as well as 1970s, were gradually phased out because they lacked specificity for heart muscle (Mythili and Malathi, 2015). The subsequent generation of biomarkers, which included CK-MB as well as LDH 1+2, was specifically targeted to heart muscle. These indicators nevertheless had an excessively elevated percentage of false-positive results, therefore

a new, better accurate biomarker are required. Although troponins was discovered for the initial time inside 1965, a trustworthy immunoassay towards measuring its concentrations within blood didn't come into existence till in the latter part of the 1990s (Danese and Montagnana, 2016). When performed 6 to 12 hours following the onset of chest discomfort, it was discovered that troponin tests had nearly 100% sensitivity and greatly enhanced specific for heart muscle injury in comparison with earlier biomarkers (Garg *et al*., 2017).

2.9.3 Kinase

Creatine kinase (CK) concentrations within the blood have often been regarded as an indirect indicator of muscle injury, notably for the identification of disorders including brain ailments, muscular dystrophy, as well as a heart attack. CK is a small enzyme with a molecular weight of 82 kDa which are present inside the mitochondria and cytoplasm of tissues with a high need for energy. Two polypeptide subunits of within 42 kDa, designated M (muscle type) as well as B (brain type), make up the cytosolic portion of CK. Three isoenzymes with tissue-specific functions can be formed thanks to these subunits: CK-BB, CK-MM, as well as CK-MB. The proportion of subunits often changes with muscle type: Cardiovascular muscle is between 70 and 80 percent MM and twenty to thirty percent MB, while the brain is mostly BB. Skeletal muscle is 98 percent MM as well as two percent MB Schlattner *et al*. (2006).

2.10 Antioxidants

2.10.1 Catalase (CAT)

The important enzyme catalase feeds on hydrogen peroxide, a nonradical ROS. The enzyme carries of neutralizing charge of hydrogen peroxide during its eleimation, hence preserving the molecule's optimal level within the cell that is also necessary for cell-to-cell communication

activities (Nandi *et al*., 2019). Catalase is one of the many important antioxidant enzymes. It is present in nearly every type of organisms that are aerobic. Catalase transforms a pair of molecules of hydrogen peroxide into a single molecule of oxygen and two molecules of water in a two-step process. The decrease of one hydrogen peroxide molecule results in the creation of a spectroscopically unique intermediate chemical I, a covalent oxyferryl species (FeIVO), with a porphyrin -cation radical (Ivancich *et al*., 1997). The second hydrogen peroxide molecule serves as an electron donor during the second step process, which reduces chemical I through redox reactions to free enzyme, oxygen, as well as water (Nandi *et al*., 2019).

2.10.2 Superoxide dismutases (SODs)

Every domains of life contain the group of metalloenzymes known as superoxide dismutases (SODs). SODs are the first line of defence against damage caused by reactive oxygen species (ROS) (Younus, 2018). These proteins reduce the quantity of superoxide anion free radical (O2-), which when present in excessive amounts harms cells (Younus, 2018), by catalyzing its dismutation into molecular oxygen as well as hydrogen peroxide (H2O2). Metal ions that exist within the active location of SODs alternately oxidize and reduce as a result of this interaction. SODs may be divided into four different categories according to the metal cofactors that are found in the active sites. Every biological kingdoms have an uneven distribution of the various SOD forms, which are found in various subcellular locations.

2.10.3 Glutathione (GSH)

GSH is important in the elimination of numerous reactive species. Yet, before we get into that, it's crucial to comprehend whence these reactive species come from as well as the pathological repercussions that GSH can help prevent (Forman *et al*., 2009). The glutathione a tripeptide of -l-

glutamyl-l-cysteinyl-glycine, is the most essential tiny molecule antioxidant produced in cells. They are produced by introducing cysteine towards glutamate as well as then glycine inside a consecutive order. The cysteine sulfhydryl group participates in reduction as well as conjugation processes, which are thought to be the most critical actions of GSH. These techniques eliminate peroxides and several xenobiotic chemicals; nevertheless, GSH implicated in life cycle of the cell control (Forman *et al*., 2009).

CHAPTER THREE

MATERIAL AND METHOD

MATERIALS AND METHODOLOGY

3.1 MATERIALS

3.1.1 PLANT MATERIAL

Fresh *Persea americana* (avocado) fruits were obtained from a local market named Oba market, Oredo Local Government of Edo state. The zest of the fruit (peels) was authenticated by the Department of plant biology and biotechnology, Faculty of Life Sciences, University of Benin, Benin City with voucher number of UBH-P408 Persea.

3.1.2 CHEMICALS

1. 100g Cadmium Chloride
2. 100g Ethylenediamine tetracetic acid (EDTA)
3. 90% alcohol (they were manufactured by Cartivalue Chemical Limited, Mumbai, India were obtained from a chemical store in Benin city).
4. Chemicals required for analysis include;
5. HNO
6. HCI
7. H_2SO_4
8. Perchloric acid

3.1.3 EXPERIMENTAL ANIMALS

They were kept in plastic enclosures in the Animal house of the Department of Animal Environmental Biology (AEB), University of Benin, Benin City, and were acclimated for two weeks beforehand the start of the experiments. A total of fifteen (15) wholesome wistar rats (males) with weight that varied from 100g towards 200g had been obtained from Pharmacology animal house, Faculty of Pharmacy.

All animals were given food (rat chow-Vital Feeds) and water ad libiru, Treatment groups were administered ethanol extract of Persea americana cadmium EDTA in addition to water and mt chow. Rats were scaled at the beginning, middle, and end of the experiment. The University of Benin's Department of Physiology's Institutional Animal Ethics Committee's requirements were followed when handling the animals.

3.1.4 MATERIALS/EQUIPMENTS REQUIRED FOR ANALYSIS

1. Animal cage
2. Oral gastric tube
3. AAS Machine
4. Dessicator
5. Rat pellet
6. Food/water tray

7. Nose mask and disposable gloves
8. .Mortal and pestle
9. Crucibles

3.2 METHODOLOGY

3.2.1 PREPARATION OF THE ETHANOLIC EXTRACT OF PERSEA AMERICANA SEEDS

The seeds of the fresh *Persea americana* purified water cleaned them after they had been painstakingly detached from their fruit, and they were then left to dry in the open for roughly three weeks. They were bace where they were incubated at 40-45% for about two hoses till they died shrunk. They were crushed into a coarne dust with mortar and pestle. They were with a mechanical grinder Oba market Oredo Local Government of Edo state. The grinded seed was weighed. It was then extracted using 90% alcohol, It was immersed in water for 72 hours while being stirred occasionally to ensure efficient extraction. The shaft was eliminated after the mixture was permitted with cheese cloth. The filtrate was obtained, put in a curable, as well as heated to a regulated temperature of 44°C for approximately 40 minutes. It was then taken out and put in a clean bottle before being chilled to four degrees Celsius in the refrigerator.

3.2.2 DOSE PREPARATION OF ETHYLENEDIAMINE TETRACETIC ACID (EDTA as STANDARD DRUG)

Al of 2000 mg of EDTA substance was weighed, then missed in 20 milliliters of distilled water and administered to the animals via oral route using an oral gastric tube. The animals were weighed before the administration to get the dose to be administered.

3.2.3 DOSE PREPARATION OF CADMIUM

A total of 1000mg of cadmium chloride was weighed as well as dissolved within 100ml of distilled and administered to the animals via oral route using oral gastric tube. The animals were weighted before the administration to get the dose to be administered.

3.2.4 DOSE PREPARATION OF PERSEA AMERICANA (AVOCADO PEAR) PEEL EXTRACT

4 spel of 2000mg of Persea americana (avocado pear) seed was weighed as well as dissolved within 20 distilled water and administered to the animals via oral route using the oral gastric tube. The e were weighed before the administration to get the dose to be administered.

3.2.5 EXPERIMENTAL DESIGN

After 14 days of acclimatization to laboratory conditions, the wistar albino rats were divided into five (5) groups of three (3) rats in each group except the control group which had five (5)

Group I served as the control and were administered distilled water.

Group II was administered lead and cadmium.

Group III was administered lead, cadmium and zinc.

Groups IV and V were administered ethanoic extract of Perseu Americana (75 mg/kg and 200 mg/kg followed concomitantly by cadmium chloride (8 mg/kg) for a period of 4 weeks. All administrations were via oral route.

3.2.6 COLLECTION OF TISSUES FOR TESTING

After the period of 28 days, the animals 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, kidney and blood was removed from selected animals, weighed and placed in a collection bottle. The samples were labelled and taken to the laboratory for analysis.

3.3 HEAVY METAL ANALYSIS

For the purpose of determining the presence of different metals, the collected samples were broken down using a wet chemical digestion technique. In the laboratory, 1 g of the samples (liver and kidney) were weighed into the digestion flask. To every part of the sample, 5 ml of perchloric acid as well as 15 ml of 01 N concentrated HNO were introducted within a ratio of 1:3, and the contents were then heated on an electric plate before the sample ended up being clear. To calculate the amount of cadmium, the contents of the flask were filtered using Whatman filter NO42 paper into a 100 ml volumetric flask, filled to the appropriate level with distilled water, and then placed in a plastic reagent bottle.

3.3.1 Nitric – perchloric acid digestion

3.3.2 Apparatus

250 ml digestion tube, Heater, Funnels, 25 ml volumetric flask, Filter paper, Beakers

3.3.3 Reagents

i. Nitric – Perchloric acid mixture. Ratio 2: 1.

ii. Nitric acid – water mixture. Ratio 1: 1.

3.3.4 Procedure

Weigh 1.0 g of dry, grind and sieved sample into the digestion tube. The combined acid is added in 10 ml. Heat the digesting tube by bringing it near the heater. When thick white fumes appear, keep heating till a clean solution is obtained. Remove off the heater, allow it cool, and then add a small quantity of deionized water. Filter the mixture using a 25 ml volumetric flask using Whitemann No. 42 filter paper. Add deionized water to achieve the desired volume. A reagent blank that matched the sample was produced lacking the sample. Determine metals of interest with Atomic absorption spectrophotometer.

3.3.5 Standard preparation (QC)

To generate a calibration curve, the apparatus (A.A.S.) was calibrated initially using Buck-certified atomic absorption standards for the relevant metals. To prevent equipment drift, a reagent blank was performed every 10 samples of analysis. To ensure precision, accuracy, and repeatability, each sample was examined three times.

3.3.6 Standard solution

10 ml stock solution (1000 ppm) is introduced into a 100 ml volumetric flask and diluted to volume with deionized water. This solution contains 100 ppm of the metal. From the diluted stock solution 10 ml of it was taken and introduced into a 100 ml volumetric flask and was made up to mark. This solution contain 10 ppm of the metal. 1, 2, 4, 6, 8, and 10 ml was taken from the second diluted stock solution in 50 ml numbered flask and then made to mark with deionized water. These solution contains 0.2, 0.4, 0.8, 1.2, 1.6, and 2.0 ppm of metal of interest.

3.4 Data Analysis

The data were expressed as means + standard error of mean. Significance of mean values of different parameters between the treatment groups and control group were analysed 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.

CHAPTER FOUR

RESULT

4.1 Effect of ethanol avocado peel extract on the total protein, uric acid, HDL, LDL, triglyceride, direct bilirubin and total bilirubin level on male albino rat.

In the determination of effect of avocado peel extract administration on total protein, uric acid, HDL, LDL, triglyceride, direct bilirubin and total bilirubin levels on albino rats are present in Table 4.6. From the result, the administration of the extract showed decrease at significant ($p < 0.05$) in total protein level on male albino rat when compared to control. In the administration of the extract showed increase at significant ($p < 0.05$) in uric acid, HDL, LDL, direct bilirubin and total bilirubin levels on male albino rat when compared to control. From the result, the administration of the extract showed decrease at significant ($p < 0.05$) in triglyceride level in all doses when compared to control except for the 10mg/ml showed increase at significant ($p < 0.05$) when compared to control.

4.2 Effect of ethanol avocado peel extract on the Creatinine, Albumin levels in the heart on male albino rat.

In the determination of effect of avocado peel extract administration on Creatinine, Albumin levels in the heart on albino rats are present in table 4.2. From the result, the administration of the extract showed increase at significant ($p < 0.05$) in Creatinine and Albumin levels on male albino rat when compared to control.

4.3 Effect of ethanol avocado peel extract on the MDA, SOD and CAT in the heart on male albino rat.

In the determination of effect of avocado peel extract administration on MDA, SOD and CAT in the heart on albino rats are present in Table 4.3. From the result, the administration of the extract showed increase at significant ($p < 0.05$) in MDA, SOD and CAT level in the heart on male albino rat when compared to control.

4.4: Effect of ethanol avocado peel on the MDA, SOD and CAT in the blood on male albino rat

In the determination of effect of avocado peel extract administration on MDA, SOD and CAT in the blood of albino rats are present in Table 4.4. From the result, the administration of the extract showed decrease at significant ($p < 0.05$) in MDA, SOD levels in the blood on male albino rat when compared to control. The administration of the avocado extract at dosage of 75 mg/ml showed increase at significant ($p < 0.05$) in CAT and levels in the blood on male albino rat when compared to control. While at dosage of 100 mg/ml showed decrease at significant ($p < 0.05$) in CAT levels in the blood on male albino rat when compared to control.

4.5: Effect of ethanol avocado peel on the MDA, SOD and CAT in the Testes on male albino rat

In the determination of effect of avocado peel extract administration on MDA, SOD and CAT in the testes on albino rats are present in Table 4.5. From the result, the administration of the extract showed decrease at significant ($p < 0.05$) in MDA in the heart on male albino rat when compared to control. From the result, the administration of the extract showed increase at significant ($p < 0.05$) in, SOD and CAT level in the heart on male albino rat when compared to control.

4.6: Levels of heavy metal present in the heart after exposure to lead and cadmium on albino rat

Levels of heavy metal present in the heart after exposure to lead and cadmium on albino rat are presented in table 4.1. The level of heavy metal present in the heart after exposure to lead and cadmium on albino rats was present in table 4.1. The concentration of lead and cadmium in control group 1 was 0.05±0.02 mg/ml and 0.03±0.01 mg/ml respectively. The concentration of lead and cadmium in group 2 was 0.05 ± 0.00 mg/ml and 0.02 ±0.00 mg/ml respectively. The concentration of lead and cadmium in zinc group 3 was 0.04±0.03 mg/ml and 0.04±0.02 mg/ml respectively. The concentration of lead and cadmium in avocado Peel group four was 0.03±0.01 mg/ml and 0.01±0.01 mg/ml respectively. The concentration of lead and cadmium in avocado Peel group five with a dosage of 400 mg/ml was 0.02±0.01 mg/ml and 0.01±0.01 mg/ml respectively. The level of heavy metal present in the heart showed significant at $p < 0.05$ decrease in lead and cadmium in group four and five.

	Pb	Cd
avocado peel 100 mg/ml	**0.02±0.01**	**0.01±0.01**
avocado peel 75 mg/ml	**0.03±0.01**	**0.01±0.01**
Control	**0.05±0.02**	**0.03±0.01**
Lead and cadmium	**0.05±0.00**	**0.02±0.00**
Zinc	**0.04±0.03**	**0.03±0.02**

Table 4.1: Levels of heavy metal present in the heart after exposure to lead and cadmium on albino rat

Table 4.2 Effect of ethanol avocado peel on the Creatinine, Albumin, levels in the heart on male albino rat.

S/N	Creat (mg/dl)	ALB(mg/dl)
avocado peel 75 mg/ml	1.36 ±0.34	2.38 ±0.84
avocado peel 100 mg/ml	1.75 ±0.50	3.72 ±0.69
Zinc	0.89 ±0.07	2.32 ±0.69
lead and cadmium	1.36 ±0.0	2.74 ±0.0
Control	1.29 ±0.38	2.26 ±0.24

Mean ± SEM * p=0.05

Table 4.3 Effect of ethanol avocado peel on the MDA, SOD, CAT in the heart on male albino rat.

S/N	MDA (Unite/ mg protein) $x10^{-5}$	SOD (Unite/ mg protein) $x10^{-3}$	CAT (Unite/ mg protein) x10
Zinc	1.97±0.91	1.96±0.59*	1.26±0.19
Control	2.03±0.82	2.20±0.42	2.07±0.00
lead and cadmium	3.82±0.35*	3.05±0.19	3.12±0.05
Avocado peel 75 mg/ml	6.80±0.23	6.38±0.06	6.19±0.04
Avocado peel 100 mg/ml	7.86±0.34	7.08±0.39	7.21±0.11

Mean ± SEM * p=0.05

Table 4.4: Effect of ethanol avocado peel on the MDA, SOD, CAT in the blood on male albino rat

S/N	MDA (Unite/ mg protein) $x10^{-7}$	SOD (Unite/ mg protein) $x10^{-4}$	CAT (Unite/ mg protein) $x10^{-3}$
Zinc	1.98±0.71	1.23±0.14	4.24±1.15
Control	6.51±0.60*	1.35±0.16*	4.91±0.78
lead and cadmium	1.49±0.31	1.88±0.03*	7.17±5.64
Avocado peel 75 mg/ml	1.22±0.39	1.13±0.04	5.52±1.11
Avocado peel 100 mg/ml	1.07±0.11	1.11±0.11*	3.18±1.02

Mean ± SEM * p=0.05

Table 4.5: Effect of ethanol avocado peel on the MDA, SOD and CAT in the Testes on male albino rat

S/N	MDA (Unite/ mg protein) $x10^{-5}$	SOD (Unite/ mg protein) $x10^{-3}$	CAT (Unite/ mg protein) x10
Zinc	4.98±2.34*	9.75±3.67*	0.91±0.41*
Control	0.84±0.23	1.07±0.08	0.06±0.01
lead and cadmium	0.94±0.19	1.70±0.59	0.10±0.04
Avocado peel 75 mg/ml	0.38±0.08	1.28±0.16	0.20±0.04
Avocado peel 100 mg/ml	0.82±0.15	2.20±0.60	0.22±0.07

Mean ± SEM * p=0.05

CHAPTER FIVE

DISCUSSION

According to the Food and Agriculture Organization of the United Nations (FAO, 2020), global agriculture produces 8.65 billion tons of food annually. During the production chain, large amounts of fruits, vegetables, cereals, and pulses are wasted, mostly after post-harvest, preparation, and domestic use (Esparza *et al*., 2020). The main agricultural wastes, including as peels, resin, and different materials, are produced every year and regularly disposed throughout the environment, causing significant health as well as ecological issues. The inexpensive antioxidant substances that could be present in these wastes include terpenes, phenolic substances, and peptides, to name a few (Lizárraga-Velázquez *et al*., 2020). The antioxidant compounds can be used as supplements to food, medicines, or medicinal items because it has been demonstrated that they play a significant role in the prevention as well as adjunctive therapy of illnesses including diabetes, cancer, hypertension, and metabolic syndrome. Environmental pollution exposure is associated with an increased likelihood of coronary artery disease. A growing body of research suggests that exposure to unnecessary metals like lead,

cadmium, as well as arsenic contributes significantly to cardiovascular disease globally, in addition to the overwhelming proof for particle airborne pollutants (Lamas *et al*., 2023).

The co-exposure of lead and cadmium which are level of heavy metal present in the heart on albino rats was present in table 4.1. The concentration of lead and cadmium in control group 1 was 0.05±0.02 mg/ml and 0.03±0.01 mg/ml respectively. The concentration of lead and cadmium in group 2 was 0.05 ± 0.00 mg/ml and 0.02 ±0.00 mg/ml respectively. The concentration of lead and cadmium in zinc group 3 was 0.04±0.03 mg/ml and 0.04±0.02 mg/ml respectively. The concentration of lead and cadmium in avocado peel group four was 0.03±0.01 mg/ml and 0.01±0.01 mg/ml respectively. The concentration of lead and cadmium in avocado peel group five with a dosage of 400 mg/ml was 0.02±0.01 mg/ml and 0.01±0.01 mg/ml respectively. The level of heavy metal present in the heart showed decrease in lead and cadmium in group four and five.

The findings of the study are in support of the study carried out by Anyanwu *et al*. (2020) who discovered that *Costus afer* protective effect against low-dose heavy metal mixture. The author reported that oral administration of *Costus afer* showed increase in triglyceride, low-density lipoprotein, and very low-density lipoprotein levels and a decrease in high-density lipoprotein.

Despite oxidative stress and decreased antioxidant metabolism may be involved, the exact mechanism by which heavy metals enhance cardiovascular risk factors is yet understood (Alissa *et al*., 2011). As a result, following exposure, avocado peel extract shows the ability to suppress lead and cadmium. Cardiovascular disease (CVD) is largely influenced by lipids along with lipoproteins, their processes of metabolism, as well transport because they control plasma cholesterol quantity, improve cholesterol acceptance through macrophages, promote foam cell development, and eventually lead to plaque and swelling. But lipoproteins as well as lipids also

play a role in cardioprotection by inhibiting the breakdown of proatherogenic compounds and reducing inflammatory protein levels.

Reverse cholesterol transfer, whereby eliminates extra cholesterol from the peripheral arteries, is greatly aided by HDL. However, HDL also possesses a number of additional advantageous biological traits that heighten its CVD protection. They involve cytoprotective, anti-inflammatory, as well as antioxidant activities (Kosmas *et al*., 2018). During the past few years, bilirubin has come to be recognized as a significant endogenous antioxidant along with anti-inflammatory chemical (Suh *et al*., 2018). According to Gul *et al*. (2014), bilirubin inhibits the growth of smooth muscle cells in the vascular system, the breakdown of low-density lipoprotein, as well as dysfunction in endothelial cells.

Total protein, uric acid, lipoprotein, triglycerides, direct bilirubin, as well as total bilirubin within the heart were all affected by co-exposure to lead and cadmium within adult albino rats. The results of the study indicated that giving the avocado extract to male albino rats resulted in lower levels of total protein and triglycerides in comparison to the control group, but greater amounts of uric acid, HDL, LDL, direct bilirubin, as well as total bilirubin throughout the heart.

According to certain research (Suh *et al*., 2018), higher bilirubin levels are related with enhanced safety versus CVD. Lower concentrations of bilirubin are linked to a lower risk of cardiovascular disease (CVD) particularly cardiovascular illness. Bilirubin has previously been shown to defend towards oxidative damage by lowering reactive oxygen species as well as may also have significant anti-atherogenetic characteristics (Ryter *et al*., 2007). However, the full range of how bilirubin operates to prevent CVD is still not fully known.

The main blood circulation protein, blood albumin, is involved in many crucial physiological processes, including preserving oncotic pressure along with microvascular integrity, controlling metabolism as well as vascular processes, acting as ligands over chemicals that it attaches to, having antioxidant properties, and having anticoagulant impacts (Chien *et al*., 2017). Creatine is a crucial component of the breakdown of energy as well as heart muscle contractions (Balestrino, 2021). The treatment of avocado peel extract showed increase at significant ($p < 0.05$) in creatinine and albumin level on male albino rat when compared to control on albino rats. The finding of this study are consistent and in support of the study carried out by Khamphaya *et al*. (2022), who discovered that the plant *Paederia foetida* possess ameliorative effects on lead exposed rats model. The author reported finding were the administration of treatment at dosage of 50, 100, 500, and 1,000 mg/kg showed exhibited significantly showed no difference in creatinine.

In the process of metabolism of energy within cells, creatine is essential. raises the amount of creatine throughout a healthy heart and is usually regarded as safe. Due to reduced creatine transporter expression as well as phosphocreatine degradation in order to avoid adenosine triphosphate (ATP) fatigue, creatine as well as phosphocreatine levels fall in cardiovascular disease (Balestrino, 2021).

Lead and cadmium poisoning caused oxidative stress, and this was accompanied by a pronounced reduction of both enzymatic as well as nonenzymatic antioxidants present in cardiac homogenates. Some of the byproducts of polyunsaturated fatty acid peroxidation within cells is malondialdehyde. A rise in free radicals leads to an excess of MDA generation. Malondialdehyde levels are frequently used as indicators of patients' state of antioxidants as well as oxidative damage (Gawe *et al*., 2004).

The effect of avocado peel extract treatment on Malondialdehyde, Superoxide dismutase, and catalase in the heart on albino rats are present in table 4.3. From the result, the treatment of the extract showed increase at significant ($p < 0.05$) in MDA, SOD and CAT level in the heart on male albino rat when compared to control. The finding of the study are consistent and in support of the study carried out by Oyinloye *et al*., (2016), discovered that *Sesamum indicum* seed possesses protective effect against cadmium toxicity. From the result of the study, the administration of treatment with doses of 200 and 400 mg/kg showed an increased the level of various enzymatic as well as nonenzymatic antioxidants level examined.

It was recently showed the antioxidant enzyme superoxide dismutase guards the heart against ischemia injury as well as inflammation. Although they have been studied, the precise methods through which SOD guards versus fibrosis as well as harm to tissues in numerous organs, particularly the heart along with lung, are still unknown. SOD is a crucial component of the cardiovascular system along with could play a role in heart shape because it functions as an antioxidant enzyme that removes superoxide as well as alters oxidant equilibrium inside tissues (Lu *et al*., 2008). The catalase, which makes up 0.025 percent of the protein within the heart's mitochondria, is crucial for detoxifying H2O2 generated by the mitochondria that serves as a vital antioxidant protection system for myocardial tissue. The increase in antioxidant enzymes that support the heart's defense mechanism demonstrated the methanol avocado extract's capacity to have cardio-protective qualities.

A potential diagnostic technique for determining oxidative stress might be the MDA measurement. One of the byproducts of polyunsaturated fatty acid peroxidation is MDA. Consequently, an elevated MDA level is a crucial sign of lipid peroxidation (Demir *et al*., 2011). A crucial barrier against the effects of oxidative stress throughout the body is provided by

superoxide dismutases. The enzyme is an effective treatment for illnesses brought on by reactive oxygen species (Younus, 2018).

The study uses the avocado peel extract to examine the lipid peroxidation processes as well as antioxidant concentrations in the blood following co-exposure to lead and cadmium in albino rats. According to the study's results, male albino rats' blood levels of antioxidant enzymes like catalase and SOD reduced significantly when compared to controls, along with their lipid peroxidation activities. Amino acids, ascorbic acid, vitamin E, microelements, as well as metabolic intermediates are the primary contributors to the antioxidant content found in human serum blood (Korotkova *et al*., 2013). Throughout human blood cells, antioxidant enzymes cooperate to combat harmful reactive oxygen species. As a result, avacodo peel extract increases lipid peroxidation along with poor antioxidant enzyme activity in the blood.

In that they are in charge of producing and transporting sperm as well as androgens, the testes function as both endocrine as well as exocrine functions (Gurung *et al*., 2023). The effect of ethanol avocado peel after treatment on the lipid peroxidation activities, and antioxidant enzymatic levels in the testes on male albino rat are present in Table 4.5. The findings of the administration of the extract presented significant reduction in MDA in the testes on male albino rat in contrast to control and also significant elevation SOD as well as catalase enzyme levels in the testes on male albino rat when compared to control. The findings of the are support of the study carried out by Jahan *et al*., (2014) that revealed the protective effects of *Ficus Religiosa* plant extract against cadmium induced oxidative damage in rat testis at dosage of 100 mg/kg. The author reported an increase catalase, SOD, GSR, protein concentrations in the testes of the rats.

Due to the testicular tissue's extremely rapid rate of division of cells as well as mitochondrial usage of oxygen as well as its substantially greater quantities of unsaturated fatty acids in comparison to other tissues, oxidative stress has become a significant contributor to the occurrence of infertility in men. Additionally, there is a fierce battle among cells for oxygen since the testicular artery is weak and the oxygen pressure remains low. As a result, the human body's antioxidant defenses cannot completely neutralize free radicals or shield the body from the negative effects of oxidative damage (Asadi *et al*., 2017). In order to increase the procedure of spermatogenesis, antioxidant usage and the creation of antioxidant treatment may disrupt through the oxidative chain reaction along with greatly enhance the body's ability to combat free radical-induced oxidative stress. As a result, the plant extract demonstrated good testicular protection by boosting the activity of antioxidants. It is logical to assume that lipid peroxidation could trigger membrane damage as well as gonadal malfunction since testicular membranes are abundant in polyenoic fatty acids, which are susceptible to peroxidative breakdown. Lipid peroxidation is a common initial sign of Cd exposure and has been related to a variety of harmful effects Cd has on biological structures. The rise in lipid peroxidation caused by Cd toxicity has been linked to changes in the system that produces antioxidants, which typically defends toward free radical toxicity with enzymes such as glutathione-S-transferase, superoxide dismutase, as well as catalase, as well as nonenzymatic molecules like glutathione (Patra *et al*., 2011). According to Winiarska-Mieczan *et al*. (2018), the primary function of antioxidants is to neutralize free radicals, prevent free radical chain reactions, as well as shield cells from the damage that they cause. The antioxidant effects of SOD, CAT, as well as glutathione transferase are tied to the body's essential defense processes. SOD, CAT, as well as glutathione reductase function are increased in reaction to short-term exposition to toxic metals, therefore

suggests that defense mechanisms as well as adaptive responses are being activated by the cells (Winiarska-Mieczan *et al*., 2018).

The presence of carotenoids, phenolics, vitamins, saponins, tannins, flavonoids, glycosides, alkaloids, phenolic compound, as well as steroids in avocado seeds contributes to their antioxidant potential (Tabeshpour *et al*., 2017). Avocado seeds act as an antioxidant by converting -ocopheroxyl radicals to -tocopherol and are high in vitamin C (Folasade *et al*., 2016). The natural polyphenol, a secondary metabolite derived from plants that has pharmacological effects on oxidative damage, metabolism of lipids, insulin resistance, as well as inflammation (Li *et al*., 2018).

Active ingredients including flavonoids, quercetin, vitamins, as well as minerals may be found in avocado peel. For the prevention of hyperlipidemia, tanin limits the digestion of fat in the gut, quercetin lowers stored fat molecules, as well as flavonoid combined polyphenol boost lipase lipoprotein function. In a nutshell, according to Van De Wier *et al*. (2017), propolis can shield internal organs. The presence of tannin, flavonoid, as well as polyphenol phytochemicals in the plant is responsible for the protective effect displayed by avocado peel against contact with cadmium and lead.

CONCLUSION

The protective effect of avocado peel against cadmium and lead toxicity, owing to its polyphenol and flavonoid content represents a potential avenue for treating heavy metal-related health conditions.

REFERENCES

Aakre, K. M. and Omland, T. (2019). Physical activity, exercise and cardiac troponins: Clinical implications. *Progress in cardiovascular diseases*, **62**(2): 108-115.

Abraham, J. D., John, A. and Jemmy, F. T. (2018). Morphological characteristics of avocado (Persea americana Mill.) in Ghana. *African Journal of Plant Science*, **12**(4): 88-97.

Adeboye, J. O., Fajonyomi, M. O., Makinde, J. M. and Taiwo, O. B. (1999). A preliminary study on the hypotensive activity of Persea americana leaf extracts in anaesthetized normotensive rats. *Fitoterapia*, **70**(1): 15-20.

Adeyemi, O. O., Okpo, S. O. and Ogunti, O. O. (2002). Analgesic and anti-inflammatory effects of the aqueous extract of leaves of Persea americana Mill (Lauraceae). *Fitoterapia*, **73**(5): 375-380.

Aggarwal, S., Qamar, A., Sharma, V. and Sharma, A. (2011). Abdominal aortic aneurysm: A comprehensive review. *Experimental & Clinical Cardiology*, **16**(1): 11.

Ahangarpour, A., Sayahi, M. and Sayahi, M. (2019). The an-tidiabetic and antioxidant properties of some phenolic phytochemicals: A review study. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 13(1): 854-857

Alissa, E. M. and Ferns, G. A. (2011). Heavy metal poisoning and cardiovascular disease. *Journal of toxicology*, 2011: 7-8.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(4): 42.

Anderson, L., Thompson, D. R., Oldridge, N., Zwisler, A. D., Rees, K., Martin, N. and Taylor, R. S. (2016). Exercise-based cardiac rehabilitation for coronary heart disease. *The Cochrane database of systematic reviews*, **2016**(1): 1-8.

Anita, B. S., Okokon, J. E. and Okon, P. A. (2005). Hypoglycemic activity of aqueous leaf extract of Persea americana Mill. *Indian Journal Pharmacology*, **37**: 525-6.

Anyanwu, B. O., Orish, C. N., Ezejiofor, A. N., Nwaogazie, I. L. and Orisakwe, O. E. (2020). Protective effect of Costus afer aqueous leaf extract (CALE) on low-dose heavy metal mixture-induced alterations in serum lipid profile and hematological parameters of male Wistar albino rats. Journal of Toxicology, 2020: 3-4

Asadi, N., Bahmani, M., Kheradmand, A. and Rafieian-Kopaei, M. (2017). The Impact of Oxidative Stress on Testicular Function and the Role of Antioxidants in Improving it: A Review. *Journal of clinical and diagnostic research : JCDR*, 11(5): 1–5

Ayala, T. and Ledesma, N. (2014) Avocado history, biodiversity and production. In Sustainable Horticultural Systems, Sustainable Development and Biodiversity; Nandwani D, (ed.). Springer International Publishing, pp. 157–205.

Baird, M. F., Graham, S. M., Baker, J. S. and Bickerstaff, G. F. (2012). Creatine-kinase-and exercise-related muscle damage implications for muscle performance and recovery. *Journal of nutrition and metabolism*, 2012: 960363

Balestrino, M. (2021). Role of creatine in the heart: Health and disease. *Nutrients*, *13*(4), 1215.

Barbosa, N. V., Nogueira, C. W., Nogara, P. A., de Bem, A. F., Aschner, M., and Rocha, J. B. T. (2017). Organoselenium compounds as mimics of selenoproteins and thiol modifier agents. *Metallomics,* **9**(12): 1703–1734.

Benjamin, E. J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S., Chiuve, S. E., Cushman, M., Delling, F. N., Deo, R., de Ferranti, S. D., Ferguson, J. F., Fornage, M., Gillespie, C., Isasi, C. R., Jiménez, M. C., Jordan, L. C., Judd, S. E., Lackland, D., Lichtman, J. H., … American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee (2018). Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. *Circulation*, **137**(12): 67–492.

Bhuyan, D.J., Alsherbiny, M.A., Perera, S., Low, M., Basu, A., Devi, O.A., Barooah, M.S., Li, C.G., Papoutsis, K. (2019). The Odyssey of Bioactive Compounds in Avocado (*Persea americana*) and Their Health Benefits. *Antioxidants*, **8**(10):426.

Birnbaum, K., DeSalle, R., Peters, C. M. and Benfey, P. N. (2003). Integrating gene flow, crop biology, and farm management in on-farm conservation of avocado (Persea americana, Lauraceae). *American Journal of Botany*, **90**(11): 1619-1627.

Brotto, M. A., Biesiadecki, B. J., Brotto, L. S., Nosek, T. M. and Jin, J. P. (2006). Coupled expression of troponin T and troponin I isoforms in single skeletal muscle fibers correlates with contractility. *American Journal of Physiology-Cell Physiology*, **290**(2): C567-C576.

Budoff, M. J., Achenbach, S., Blumenthal, R. S., Carr, J. J., Goldin, J. G., Greenland, P. and Wiegers, S. E. (2006). Assessment of coronary artery disease by cardiac computed tomography: a scientific statement from the American Heart Association Committee on Cardiovascular Imaging and Intervention, Council on Cardiovascular Radiology and Intervention, and Committee on Cardiac Imaging, Council on Clinical Cardiology. *Circulation*, **114**(16): 1761-1791.

Campose, H., Khoo, C. and Sacks, F. M. (2005). Diurnal and acute pattern of postprandial apolipoprotein B-48 in VLDL, IDL and LDL from normolipidemic human. *Atherosclerosis*, **181**(2): 345-351.

Cervantes-Paz, B. and Yahia, E. M. (2021). Avocado oil: Production and market demand, bioactive components, implications in health, and tendencies and potential uses. *Comprehensive Reviews in Food Science and Food Safety*, **20**(4): 4120-4158.

Chien, S. C., Chen, C. Y., Lin, C. F. and Yeh, H. I. (2017). Critical appraisal of the role of serum albumin in cardiovascular disease. *Biomarker research*, 5: 1-9.

Colombo, R. and Papetti, A. (2019). Avocado (Persea ameri-cana Mill.) by-products and their impact: from bioactive compounds to biomass energy and sorbent material for remo-ving contaminants. A review. *International Journal of Food Science & Technology*, 54(4): 943-951.

Cowan, A.K. and Wolstenholme, B.N. (2016) Avocado. In: Caballero B., Finglas P.M. and Toldrá F. (eds.) *Encyclopedia of Food and Health.* Academic Press; Oxford, UK. pp. 294–300.

Danese, E. and Montagnana, M. (2016). An historical approach to the diagnostic biomarkers of acute coronary syndrome. *Annals of translational medicine*, **4**(10): 194.

De Almeida, A. P., Miranda, M. M. F. S., Simoni, I. C., Wigg, M. D., Lagrota, M. H. C. and Costa, S. S. (1998). Flavonol monoglycosides isolated from the antiviral fractions of Persea americana (Lauraceae) leaf infusion. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **12**(8): 562-567.

De Backer, G., Ambrosioni, E., Borch-Johnsen, K., Brotons, C., Cifkova, R., Dallongeville, J. and Wood, D. (2004). Erratum: European guidelines on cardiovascular disease prevention in clinical practice (Atherosclerosis (2003) 171 (145-155. Atherosclerosis, **173**(2): 379-391.

Demir, F., Uzun, F. G., Durak, D. and Kalender, Y. (2011). Subacute chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin. *Pesticide Biochemistry and Physiology*, 99(1): 77-81.

Duda-Chodak, A. and Tarko, T. (2007). Antioxidant properties of different fruit seeds and peels. *Acta Scientiarum Polonorum Technologia Alimentaria*, 6(3): 29-36.

Duester K.C. (2000). Avocados a look beyond basic nutrition for one of nature's whole foods. *Nutrition Today's,* **35**:151–157.

Dunbar, S.B., Khavjou, O.A., Bakas, T., Hunt, G., Kirch, R.A., Leib, A.R., Morrison, R.S., Poehler, D.C., Roger, V.L. and Whitsel, L.P. (2018) American Heart Association. Projected Costs of Informal Caregiving for Cardiovascular Disease: 2015 to 2035: A Policy Statement From the American Heart Association. *Circulation*, **137**(19):e558-e577

Esparza, I., Jiménez-Moreno, N., Bimbela, F., Ancín-Azpilicueta, C. and Gandía, L. M. (2020). Fruit and vegetable waste management: Conventional and emerging approaches. *Journal of environmental management*, *265*, 110510.

FAO, (2020). Strategic Work of FAO for Sustainable Food and Agriculture

Farley, A., McLafferty, E. and Hendry, C. (2012). The cardiovascular system. *Nursing standard (Royal College of Nursing (Great Britain):* **27**(9): 35–39

Felner, J. M. (1990). An Overview of the Cardiovascular System. In: Walker, H.K., Hall, W.D. and Hurst, J.W, (eds.) *Clinical Methods: The History, Physical, and Laboratory Examinations*. 3rd Edition. Butterworths; Boston: pp.23-34.

Folasade, O. A., Olaide, R. A. and Olufemi, T. A. (2016). Antioxidant properties of Persea americana M. seed as affected by different extraction solvent. *Journal of Advances in Food Science & Technology*, *3*(2): 101-106.

Forman, H. J., Zhang, H. and Rinna, A. (2009). Glutathione: overview of its protective roles, measurement, and biosynthesis. *Molecular aspects of medicine*, **30**(1-2): 1–12.

Fox, C. S., Pencina, M. J., Wilson, P. W., Paynter, N. P., Vasan, R. S. and D'Agostino Sr, R. B. (2008). Lifetime risk of cardiovascular disease among individuals with and without diabetes stratified by obesity status in the Framingham heart study. *Diabetes care*, **31**(8): 1582-1584.

Galindo-Tovar, M. E., Arzate-Fernández, A. M., Ogata-Aguilar, N. and Landero-Torres, I. (2007). The avocado (Persea americana, Lauraceae) crop in Mesoamerica: 10,000 years of history. *Harvard papers in botany*, **12**(2): 325-334.

Garg, P., Morris, P., Fazlanie, A. L., Vijayan, S., Dancso, B., Dastidar, A. G. and Haaf, P. (2017). Cardiac biomarkers of acute coronary syndrome: from history to high-sensitivity cardiac troponin. *Internal and emergency medicine*, **12**: 147-155.

Gaweł, S., Wardas, M., Niedworok, E. and Wardas, P. (2004). Dialdehyd malonowy (MDA) jako wskaźnik procesów peroksydacji lipidów w organizmie [Malondialdehyde (MDA) as a lipid peroxidation marker]. *Wiadomosci lekarskie (Warsaw, Poland : 1960)*, 57(9-10): 453–455.

Goff Jr, D. C., Lloyd-Jones, D. M., Bennett, G., Coady, S., D'agostino, R. B., Gibbons, R. and Wilson, P. W. (2014). 2013 ACC/AHA guideline on the assessment of cardiovascular

risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*, **129**(25): S49-S73.

Greenland, P., Alpert, J.S., Beller, G.A., Benjamin, E.J., Budoff, M.J., Fayad, Z.A., Foster, E., Hlatky, M.A., Hodgson, J.M., Kushner, F.G., Lauer, M.S., Shaw, L.J., Smith, S.C., Taylor, A.J., Weintraub, W.S., Wenger, N.K., Jacobs, A.K., Smith, S.C., Anderson, J.L., Albert, N., Buller, C.E., Creager, M.A., Ettinger, S.M., Guyton, R.A., Halperin, J.L., Hochman, J.S., Kushner, F.G., Nishimura, R., Ohman, E.M., Page, R.L., Stevenson, W.G., Tarkington, L.G. and Yancy, C.W. American College of Cardiology Foundation. American Heart Association. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Journal of the American College of Cardiology*, **56**(25): 50-103

Gul, M., Kalkan, A. K. and Uyarel, H. (2014). Serum bilirubin: a friendly or an enemy against cardiovascular diseases?. *Journal of critical care*, 29(2): 305-306.

Gurung P, Yetiskul E, Jialal I. Physiology, Male Reproductive System. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 J

Hand, P. J., Haisma, J. A., Kwan, J., Lindley, R. I., Lamont, B., Dennis, M. S. and Wardlaw, J. M. (2006). Interobserver agreement for the bedside clinical assessment of suspected stroke. *Stroke*, **37**(3): 776-780.

Harris, C., Croce, B. and Cao, C. (2016). Thoracic aortic aneurysm. *Annals of cardiothoracic surgery*, **5**(4): 407.

Ivancich, A., Jouve, H. M., Sartor, B. and Gaillard, J. (1997). EPR investigation of compound I in Proteus mirabilis and bovine liver catalases: formation of porphyrin and tyrosyl radical intermediates. *Biochemistry*, **36**(31): 9356-9364.

Jacob, R. and Khan, M. (2018). Cardiac biomarkers: what is and what can be. *Indian Journal of Cardiovascular Disease in Women-WINCARS*, **3**(04): 240-244.

Jahan, S., Zahra, A., Irum, U., Iftikhar, N. and Ullah, H. (2014). Protective effects of different antioxidants against cadmium induced oxidative damage in rat testis and prostate tissues. Systems biology in reproductive medicine, 60(4): 199-205.

Kavousi, M., Leening, M. J., Nanchen, D., Greenland, P., Graham, I. M., Steyerberg, E. W. and Franco, O. H. (2014). Comparison of application of the ACC/AHA guidelines, Adult Treatment Panel III guidelines, and European Society of Cardiology guidelines for cardiovascular disease prevention in a European cohort. *Jama*, **311**(14): 1416-1423.

Khafaji, H. A. H. and Al Suwaidi, J. M. (2014). Atypical presentation of acute and chronic coronary artery disease in diabetics. *World journal of cardiology*, **6**(8): 802.

Khamphaya, T., Pouyfung, P., Yimthiang, S., Kotepui, M. and Kuraeiad, S. (2022). Ameliorative effects of Paederia foetida Linn. on lead acetate-exposed rats. Journal of Applied Pharmaceutical Science, 12(3): 160-170.

Kim, H. W., Murakami, A., Nakamura, Y. and Ohigashi, H. (2002). Screening of edible Japanese plants for suppressive effects on phorbol ester-induced superoxide generation in differentiated HL-60 cells and AS52 cells. *Cancer letters*, **176**(1), 7-16.

Kirchberger, I., Meisinger, C., Heier, M., Kling, B., Wende, R., Greschik, C. and Kuch, B. (2011). Patient-reported symptoms in acute myocardial infarction: differences related to ST-segment elevation: the MONICA/KORA Myocardial Infarction Registry. *Journal of internal medicine*, **270**(1): 58-64.

Korotkova, E. I., Freinbichler, W., Linert, W., Dorozhko, E. V., Bukkel, M. V., Plotnikov, E. V. and Voronova, O. A. (2013). Study of total antioxidant activity of human serum blood in the pathology of alcoholism. *Molecules* (Basel, Switzerland), 18(2): 1811–1818.

Kosińska, A., Karamać, M., Estrella, I., Hernández, T., Bartolomé, B. and Dykes, G.A. (2012). Phenolic compound profiles and antioxidant capacity of *Persea americana Mill*. peels and seeds of two varieties. *Journal of Agricultural and Food Chemistry*, 60(18): 4613-4619.

Kosmas, C. E., Martinez, I., Sourlas, A., Bouza, K. V., Campos, F. N., Torres, V., Montan, P. D. and Guzman, E. (2018). High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. *Drugs in context*, 7: 212525.

Lamas, G. A., Bhatnagar, A., Jones, M. R., Mann, K. K., Nasir, K., Tellez-Plaza, M. and American Heart Association Council on Epidemiology and Prevention; Council on Cardiovascular and Stroke Nursing; Council on Lifestyle and Cardiometabolic Health; Council on Peripheral Vascular Disease; and Council on the Kidney in Cardiovascular Disease. (2023). Contaminant Metals as Cardiovascular Risk Factors: A Scientific Statement From the American Heart Association. *Journal of the American Heart Association*, pp.2-9.

Li, S., Tan, H. Y., Wang, N., Cheung, F., Hong, M. and Feng, Y. (2018). The potential and action mechanism of polyphenols in the treatment of liver diseases. *Oxidative medicine and cellular longevity*, 2018: 2-6.

Litz, R. E., Witjaksono, W., Raharjo, S., Efendi, D., Pliego-Alfaro, F. and Barceló-Munoz, A. (2005). Persea americana avocado. In *Biotechnology of fruit and nut crops.* Wallingford UK: CAB International. pp. 326-947.

Lizárraga-Velázquez, C. E., Leyva-López, N., Hernández, C., Gutiérrez-Grijalva, E. P., Salazar-Leyva, J. A., Osuna-Ruíz, I., ... & Ávalos-Soriano, A. (2020). Antioxidant molecules from plant waste: Extraction techniques and biological properties. *Processes*, *8*(12): 1566.

Lloyd-Jones, D. M., Larson, M. G., Beiser, A. and Levy, D. (1999). Lifetime risk of developing coronary heart disease. *The Lancet*, **353**(9147): 89-92.

Lu, Z., Xu, X., Hu, X., Zhu, G., Zhang, P., Van Deel, E. D. and Chen, Y. (2008). Extracellular superoxide dismutase deficiency exacerbates pressure overload–induced left ventricular hypertrophy and dysfunction. *Hypertension*, 51(1): 19-25.

Mirkin, J., Radecki, R. and Spiegel, R. (2019). Deriving Peace of Mind: In Search of a Fifth-Generation Troponin Testing Threshold to Safely Rule Out Acute Myocardial Infarction:

March 2019 Annals of Emergency Medicine Journal Club. *Annals of Emergency Medicine*, **73**(3): 317-319.

Morton JF (1987). Fruits of warm climates. Julia F. Morton, Miami, Florida, 505p.

Mythili, S. and Malathi, N. (2015). Diagnostic markers of acute myocardial infarction. *Biomedical reports*, **3**(6): 743-748.

Nandi, A., Yan, L. J., Jana, C. K. and Das, N. (2019). Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. *Oxidative medicine and cellular longevity*, **2019**: 13-90.

National Cholesterol Education Program (NCEP). (2002). Expert panel on detection, evaluation and treatment of high blood in adults (adult treatment panel III) final report. Circulation. 106:3143–3421.

Nayak, B. S., Raju, S. S. and Chalapathi Rao, A. V. (2008). Wound healing activity of Persea americana (avocado) fruit: a preclinical study on rats. *Journal of wound care*, **17**(3): 123-125.

Nigam P. K. (2011). Serum Lipid Profile: Fasting or Non-fasting? *Indian journal of clinical biochemistry: IJCB*, **26**(1): 96–97.9

Nwauzoma, A. B. and Dappa, M. S. (2013). Ethnobotanical studies of port Harcourt metropolis, Nigeria. *International Scholarly Research Notices*, 2013: 2-9.

Ogunwusi, A. A. and Ibrahim, H. D. (2016). Economic significance of avocado pear in Nigeria. *Developing country studies*, **6**(3): 1-10.

Ojewole, J. A. and Amabeoku, G. J. (2006). Anticonvulsant effect of Persea americana Mill (Lauraceae)(Avocado) leaf aqueous extract in mice. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **20**(8): 696-700.

Owolabi, M. A., Jaja, S. I. and Coker, H. A. (2005). Vasorelaxant action of aqueous extract of the leaves of Persea americana on isolated thoracic rat aorta. *Fitoterapia*, **76**(6): 567-573. 9

Oyinloye, B. E., Ajiboye, B. O., Ojo, O. A., Nwozo, S. O. and Kappo, A. P. (2016). Cardioprotective and Antioxidant Influence of Aqueous Extracts from Sesamum indicum Seeds on Oxidative Stress Induced by Cadmium in Wistar Rats. Pharmacognosy magazine, 12(2): 170–174.

Patra, R. C., Rautray, A. K. and Swarup, D. (2011). Oxidative stress in lead and cadmium toxicity and its amelioration. *Veterinary medicine international*, 2011: 4-7.

Pliego-Alfaro, F. and Litz, R. E. (2007). Biotechnology and avocado improvement. *California Avocado Society*, **90**: 149-167.

Popenoe, W. and Zentmyer, G. A. (1963). Early history of the avocado. *Calif. Avocado Soc. Yearbook*, **47**: 19-24.

Potocki, M., Reichlin, T., Thalmann, S., Zellweger, C., Twerenbold, R., Reiter, M. and Mueller, C. (2012). Diagnostic and prognostic impact of copeptin and high-sensitivity cardiac troponin T in patients with pre-existing coronary artery disease and suspected acute myocardial infarction. *Heart*, **98**(7): 558-565.

Potter, J. M., Hickman, P. E. and Cullen, L. (2022). Troponins in myocardial infarction and injury. *Australian Prescriber*, **45**(2): 53.

Qin, R., Wu, Y., Xu, Z., Xie, D. and Zhang, C. (2013). Assessing the impact of natural and anthropogenic activities on groundwater quality in coastal alluvial aquifers of the lower Liaohe River Plain, NE China. *Applied Geochemistry*, **31**: 142-158.

Rahmani, G., Martin-Smith, J. and Sullivan P. (2017). The Avocado Hand. *Irish Medical Journal,* **110**:658.

Rasmussen, M. and Jin, J. P. (2021). Troponin variants as markers of skeletal muscle health and diseases. *Frontiers in Physiology*, **12**: 747214.

Ryter, S. W., Morse, D. and Choi, A. M. (2007). Carbon monoxide and bilirubin: potential therapies for pulmonary/vascular injury and disease. *American journal of respiratory cell and molecular biology*, 36(2): 175-182.

Sadiye, A. K. A. N. (2021). Phytochemicals in avocado peel and their potential uses. *Food and Health*, 7(2): 138-149.

Savji, N., Rockman, C. B., Skolnick, A. H., Guo, Y., Adelman, M. A., Riles, T. and Berger, J. S. (2013). Association between advanced age and vascular disease in different arterial territories: a population database of over 3.6 million subjects. *Journal of the American College of Cardiology*, **61**(16): 1736-1743.

Savji, N., Rockman, C. B., Skolnick, A. H., Guo, Y., Adelman, M. A., Riles, T. and Berger, J. S. (2013). Association between advanced age and vascular disease in different arterial territories: a population database of over 3.6 million subjects. *Journal of the American College of Cardiology*, **61**(16): 1736-1743.

Schaffer, B., Wolstenholme, B.N. and Whiley, A.W. (2013). The avocado: botany, production and uses. CABI. 560p.

Schlattner, U., Tokarska-Schlattner, M. and Wallimann, T. (2006). Mitochondrial creatine kinase in human health and disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, **1762**(2): 164-180.

Searls, D. E., Pazdera, L., Korbel, E., Vysata, O. and Caplan, L. R. (2012). Symptoms and signs of posterior circulation ischemia in the new England medical center posterior circulation registry. *Archives of neurology*, **69**(3): 346-351.

Setyawan, H. Y., Sukardi, S. and Puriwangi, C. A. (2021). Phytochemicals properties of avocado seed: A review. In *IOP Conference Series: Earth and Environmental Science,* IOP Publishing. 733(1): 2-9.

Sharp, A. L., Baecker, A. S., Shen, E., Redberg, R., Lee, M. S., Ferencik, M. and Sun, B. C. (2019). Effect of a HEART care pathway on chest pain management within an integrated health system. *Annals of emergency medicine*, **74**(2): 171-180.

Shil, S., Choudhury, M. D., & Das, S. (2014). Indigenous knowledge of medicinal plants used by the Reang tribe of Tripura state of India. *Journal of ethnopharmacology*, 152(1): 135-141.

Siket, M. S. and Edlow, J. (2013). Transient ischemic attack: an evidence-based update. *Emergency Medicine Practice,* **15**(1): 1-26.

Sontheimer, D. L. (2006). Peripheral vascular disease: diagnosis and treatment. *American family physician*, **73**(11): 1971-1976.

Suh, S., Cho, Y. R., Park, M. K., Kim, D. K., Cho, N. H. and Lee, M. K. (2018). Relationship between serum bilirubin levels and cardiovascular disease. *PLoS One*, *13*(2): 1-9.

Swap, C. J. and Nagurney, J. T. (2005). Value and limitations of chest pain history in the evaluation of patients with suspected acute coronary syndromes. *Jama*, **294**(20): 2623-2629.

Tabeshpour, J., Razavi, B. M. and Hosseinzadeh, H. (2017). Effects of avocado (Persea americana) on metabolic syndrome: A comprehensive systematic review. *Phytotherapy research*, *31*(6): 819-837.

Taulavuori, K., Julkunen-Tiitto, R., Hyöky, V. and Taulavuori E. (2013). Blue Mood for Superfood. *Natural Product Communications,* 8:19-45.

Thaulow, E., Erikssen, J., Sandvik, L., Erikssen, G., Jorgensen, L. and Colin, P. F. (1993). Initial clinical presentation of cardiac disease in asymptomatic men with silent myocardial ischemia and angiographically documented coronary artery disease (the Oslo Ischemia Study). *The American journal of cardiology*, **72**(9): 629-633.

Thaulow, E., Erikssen, J., Sandvik, L., Erikssen, G., Jorgensen, L. and Colin, P. F. (1993). Initial clinical presentation of cardiac disease in asymptomatic men with silent myocardial ischemia and angiographically documented coronary artery disease (the Oslo Ischemia Study). *The American journal of cardiology*, **72**(9): 629-633.

Ukwe, C. V. and Nwafor, S. V. (2004). Anti-ulcer activity of aqueous leaf extract of Persea americana (family-Lauraceae). *Nigerian Journal of Pharmaceutical Research*, *3*(1): 91-95.

Van De Wier, B., Koek, G. H., Bast, A. and Haenen, G. R. (2017). The potential of flavonoids in the treatment of non-alcoholic fatty liver disease. *Critical reviews in food science and nutrition*, *57*(4): 834-855.

Vasan, R. S., Sullivan, L. M., Wilson, P. W., Sempos, C. T., Sundström, J., Kannel, W. B. and D'agostino, R. B. (2005). Relative importance of borderline and elevated levels of coronary heart disease risk factors. *Annals of internal medicine*, **142**(6): 393-402.

Vasan, R. S., Sullivan, L. M., Wilson, P. W., Sempos, C. T., Sundström, J., Kannel, W. B. and D'agostino, R. B. (2005). Relative importance of borderline and elevated levels of coronary heart disease risk factors. *Annals of internal medicine*, **142**(6): 393-402.

Wei, B. and Jin, J. P. (2016). TNNT1, TNNT2, and TNNT3: Isoform genes, regulation, and structure–function relationships. *Gene*, **582**(1): 1-13.

Winiarska-Mieczan A. (2018). Protective effect of tea against lead and cadmium-induced oxidative stress-a review. Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine, 31(6): 909–926.

Wolfe, K. L. and Liu, R. H. (2003). Apple peels as a value-added food ingredient. *Journal of Agricultural and Food Chemistry*, 51(6): 1676-1683.

Wu, A. H. and Feng, Y. J. (1998). Biochemical differences between cTnT and cTnI and their significance for diagnosis of acute coronary syndromes. *European heart journal*, 19: 9-25.

Xu, R. Y., Zhu, X. F., Yang, Y. and Ye, P. (2013). High-sensitive cardiac troponin T. *Journal of geriatric cardiology: JGC*, **10**(1): 102.

Yahia, E. M. and Woolf, A. B. (2011). Avocado (Persea americana Mill.). Postharvest Biology and Technology of Tropical and Subtropical Fruits, Mangosteen to white sapote. *Elsevier*, pp.125–186.

Yasir, M., Das, S. and Kharya, M. D. (2010). The phytochemical and pharmacological profile of Persea americana Mill. *Pharmacognosy reviews*, **4**(7): 77.

Younus H. (2018). Therapeutic potentials of superoxide dismutase. *International journal of health sciences*, 12(3): 88–93.

Younus, H. (2018). Therapeutic potentials of superoxide dismutase. *International journal of health sciences*, **12**(3): 88.

Yusuf, S., Hawken, S., Ounpuu, S., Dans, T., Avezum, A., Lanas, F., McQueen, M., Budaj, A., Pais, P., Varigos, J. and Lisheng, L. (2004). INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*, **364**(9438):937-52.

Zhao, L., Zong, W., Zhang, H. and Liu, R. (2019). Kidney toxicity and response of selenium containing protein-glutathione peroxidase (3) to CdTe QDs on different levels. *Toxicological Sciences*, **168**(1): 201-208.