ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS OF *Phyllanthus amarus* IN CARRAGEENAN INDUCED INFLAMMATION IN MICE

BY

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DECLARATION

I hereby declare that this project has been written by me and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other University. All citations and sources of information are clearly acknowledged by means of reference.

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UDEAGHA CHIDERA LESLY Date

CERTIFICATION

This is to certify that this project report titled **'Anti-inflammatory and Antioxidant Effects of Phyllanthus Amarus in Carrageenan Induced Inflammation in Mice'** was prepared and submitted b**y** UDEAGHA Chidera lesly with matriculation number 17010102017 of Biochemistry in the department of Biological sciences, in partial fulfilment of the requirements for the award of Bachelor of science (B.Sc) degree in Biochemistry.

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DEDICATION

This research project is dedicated to GOD Almighty, the Alpha and the Omega for his grace upon my life, for his love, for his guidance, for being my solid rock, for being my father and for seeing me through this research work.

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ABSTRACT

Inflammation is a pathological state that develops from immunological, vascular, and inflammatory cell responses to infection or damage, and is characterized at the tissue level by redness, swelling, heat, discomfort, and loss of tissue function. This can result in DNA damage, tissue death, and interior scarring, all of which have been related to the onset of a variety of disorders. The difference between the formation of reactive oxygen species (ROS) and their elimination by protective systems is known as oxidative stress, and it can lead to chronic inflammation. Many chronic diseases are caused by oxidative stress-induced inflammation. The effects of P. amarus extract on paw oedema, lipid profile, and antioxidant capabilities in carrageenan-induced inflammatory mice were investigated in this work. For seven days, forty-five mice were separated into nine groups and given varied concentrations of P. amarus extract and fractions. Normal saline was administered to the normal control mice, whereas the positive control mice were given the standard medication (Diclofenac). Using Randox kits, the lipid profile and amounts of antioxidant enzymes [catalase, superoxide dismutase (SOD), and reduced glutathione (GSH)] were measured in the mice's serum and liver. P. amarus extract reduced serum and liver total cholesterol, triglycerides, VLDL-C, and LDL-C significantly ($p< 0.05$), whereas serum HDL-C was significantly raised. SOD, Catalase activity, and Glutathione concentration all increased significantly ($p<0.05$) in P. amarus.This study showed that *P. amarus* exerts a hypolipidemic and antioxidant effects which improves the lipid profile of the inflammatory mice.

KEY WORDS: Antioxidant, Inflammation, Lipid profile, *P . amarus,* Oxidative stress.

CHAPTER ONE
INTRODUCTION

1.1 **Background to the Study**

Phyllanthus amarus is a weed that is widely known for its medicinal properties. It belongs to the Euphorbiaceae family and is also known as wind gale (Joseph et al. 2011). P. amarus is a unique anti-inflammatory therapeutic agent that is used to treat jaundice, diarrhoea, dysentery, wounds, ulcers, kidney disorders, diabetes, viral hepatitis, and urogenital diseases, as well as being used to treat jaundice, diarrhoea, dysentery, wounds, ulcers, kidney disorders, diabetes, viral hepatitis, and urogenital diseases (Calixto, 2000). Plants in the *Phyllanthus* genus can be found in practically every tropical and subtropical countries, and they've long been used to treat chronic liver disease in traditional medicine (Lui et al. 2003). *P.amarus* is known as "eyin olobe" in the Yoruba part of Nigeria, where it is used as an ingredient in "agbo" and the infusion of its leaves is used for haemorrhoids (swollen and inflamed veins in the rectum or anus). It is also known as "Ebebenizo" in Benin, where it is used to relieve pain, remove gas from the intestines, stimulate and facilitate digestion, and it also acts as mild laxative (Etta, 2008).

With an average of 2000 mg/kg/day, *P. amarus* has been determined to have a minimal potential for toxicity (Krithika et al. 2009). The presence of flavonoids, tannins, alkaloids, and saponins in *P. amarus* was confirmed by phytochemical investigation (Naaz et al. 2007). *P. amarus* has antibacterial, antiviral, chemoprotective, antimutagenic, and hypoglycemic characteristics, and it is utilized for its hepatoprotective, anti-tumor, anti-diabetic, anti-hypertensive, analgesic, anti-inflammatory, and anti-microbial properties (Joseph et al. 2011).

The extract of P. amarus was discovered to have a robust and effective non-concentration dependent inhibitory impact on the development of calcium oxalate crystals, the building blocks of most kidney stones, and this response has a very high pathogenic concentration, which explains why it has been used in traditional medicine as a prevention against kidney stone formation (Calixto, 2000).

Inflammation is the immune system's reaction to adverse stimuli such as pathogens, damaged cells, poisonous substances, or irradiation, and it works by removing harmful stimuli and starting the healing process (Ferrero et al. 2007).

Redness, swelling, heat, pain, and loss of tissue function are all symptoms of inflammation, and they are caused by local immune, vascular, and inflammatory cell responses to infection or injury (Takeuchi et al. 2010).

Toxins can cause inflammation, which is an immune system response. Toxic chemicals can cause tissue damage or disease by triggering acute and chronic inflammatory reactions in the heart, pancreas, liver, kidneys, lungs, brain, intestines, and reproductive system (Takeuchi et al. 2010). Inflammation is treated differently depending on the source and degree; in some circumstances, no treatment is required; however, during an allergic reaction, inflammation can cause substantial swelling that closes the airways, making breathing hard, and it is vital to treat it (Felman et al. 2020).

In this study, Carrageenan was used to induce inflammation in mice thereby testing for the anti-inflammatory and anti-oxidant effects of *P. amarus* in inflammation in order to investigate how *P. amarus* could serve as a medicinal therapeutic agent in treatment of inflammation and related diseases.

1.2 Statement of the problem

Inflammation is a pathological condition characterized by redness, swelling, heat, pain, and loss of tissue function at the tissue level. It is caused by local immune, vascular, and inflammatory cell responses to infection or injury, and can result in DNA damage, tissue death, and internal scarring, all of which have been linked to the development of several diseases, including cancer (Takeuchi et al. 2010). Traditional medicine has been employed to treat a variety of disorders in recent years, owing to the low toxicity of herbal medications (Takeuchi et al. 2010). Using carrageenan to induce inflammation in mice, we will be testing for the antioxidant and anti-inflammatory effects of *P. amarus* and to also confirm that *P.amarus* can serve as a therapeutic agent to treat inflammation.

1.3 Aim and objectives of the study

This research project aims to investigate the anti-inflammatory and antioxidant effects of the hydro-ethanol extract of *P. amarus* in carrageenan induced inflammation in mice.

The specific objectives are to:

i. Determine the lipid profile of carrageenan induced active inflammation in mice.
ii. Determine the effect of hydroethanol extract of *P. amarus* on Lipid peroxidation in carrageenan induced inflammatory in mice.

iii. Determine the effect of hydroethanol extract of *P. amarus* on the activities of antioxidant enzymes (Superoxide dismutase and catalase) in carrageenan induced inflammation in mice.
iv. Determine the effect of hydroethanol extract of *P. amarus* on the levels of reduced Glutathione (GSH) in carrageenan induced inflammation in mice.

1.4 Scope of the study

This study is an *in vivo* study that involves working on typical animal models such as mice. Using carrageenan to induce inflammation in mice, we will be testing for the antioxidant and anti-inflammatory effects of *P. amarus*. *In vivo* study evaluates the effects of diverse biological entities on entire, living organisms or cells, mainly animals, including humans, and plants (Flanagin et al. 2007).

1.5 Significance of the study

The importance of this study is to test for the presence of anti-inflammatory and antioxidant effects of *P. amarus* in inflammation and also to investigate on *P. amarus* to be used as a therapeutic agent in the treatment of inflammation. *P. amarus* has been reported to possess anti-inflammatory and antioxidant activity. In this context it is expedient to confirm that *P. amarus* can be used as a therapeutic agent in the treatment of inflammation and other related diseases.

1.6 Definition of terms

- Superoxide dismutase- is a biological enzyme that aids in the breakdown of potentially harmful oxygen molecules, potentially preventing tissue injury.
- Toxicology- is the ability of a chemical (a toxin or poison) to harm humans or animals.
- Inflammation- is a defense mechanism employed by the body's white blood cells and the substances they generate to protect a person from outside invaders such as bacteria and viruses.
- Carrageenan induced inflammation - It has significant predictive value for anti-inflammatory medicines acting through acute inflammation mediators because it is beneficial in detecting orally active anti-inflammatory medications.

- Lipid peroxidation- A series of oxidative lipid breakdown events is known as lipid peroxidation. By stealing electrons from lipids in cell membranes, free radicals harm cells. This activity is caused by a chain reaction of free radicals.
- Glutathione- catalyzes the conversion of hydrogen peroxide to water and oxygen, as well as the conversion of peroxide radicals to alcohols and oxygen, in the cytosol.
- A lipid profile- is a collection of blood tests used to detect abnormalities in lipids such as cholesterol and triglycerides.

CHAPTER TWO
LITERATURE REVIEW

2.1 *Phyllanthus amarus*

Phyllanthus amarus is a weed plant that is well recognized for its therapeutic benefits. It is a member of the Euphorbiaceae family and is also known as gale of wind (Raj et al. 2011). P. amarus is used to treat jaundice, diarrhoea, dysentery, wounds, ulcers, renal disorders, diabetes, viral hepatitis, and urogenital diseases, as well as being an unique anti-inflammatory drug (Calixto, 2000). Plants in the genus P. amarus can be found in most tropical and subtropical areas, and have long been used in traditional medicine to treat chronic liver illness (Lui et al. 2003). In the Yoruba part of Nigeria *P. amarus* is called "eyin olobe" and it is used as an ingredient of "agbo" and the infusion of its leaves is used for haemorrhoid (swollen and inflamed veins in the rectum or anus), in Igbo tribe it is called "ngwu" and it is called "Ebebenizo" in Benin and it is generally used to relieve pain, remove gas from the intestines, stimulate and facilitate digestion, as an anti-helminthes to expel intestinal worms, and it acts as a mild laxative (Etta, 2008). *P. amarus* has been reported to be used as a therapeutic agent in treating problems of stomach, genitourinary system, liver, kidney and spleen. It is bitter, astringent, stomachic, diuretic, febrifuge and antiseptic (Chevallier, 2000).

With an average of 2000 mg/kg/day, *P. amarus* has been determined to have a minimal potential for toxicity (Krithika et al. 2009). The presence of flavonoids, tannins, alkaloids, and saponins in *P. amarus* was confirmed by phytochemical investigation (Naaz et al. 2007). *P.amarus* has antibacterial, antiviral, chemoprotective, antimutagenic, and hypoglycemic characteristics, and it is utilized for its hepatoprotective, anti-tumor, anti-diabetic, anti-hypertensive, analgesic, anti-inflammatory, and anti-microbial properties (Joseph et al. 2011).

P. *amarus* was found to have traditional uses for kidney stones and gall bladder stones, where the extract of this plant was discovered to have a robust and effective non-concentration dependent inhibitory impact on the development of calcium oxalate crystals and the building blocks of most kidney stones, and this response has a very high pathogenic concentration which explains why it has been used in traditional medicine as prevention against kidney stone formation (Calixto, 2002).

The leaves of *P. amarus* were reported to include nutritious components such crude lipids, protein, glucose, fiber, and anti-nutrient components like oxalate and phytate. Protein, fat,

ash, fiber, and carbohydrate are all present in *P. amarus*, as well as mineral components such as iron, manganese, magnesium, zinc, calcium, potassium, phosphorus, copper, and chromium using the standard methods of the association of official analytical chemists (AOAC, 2000).

According to proximate analysis, both the leaf and the seed are rich in carbohydrate and calories, low in fat, ash, crude fiber, moderate in protein, and have an appropriate moisture level for preservation (Umofia et al. 2017). The leaf has significantly higher protein and ash content than the seed, but lower carbohydrate and calorie content, with no discernible difference in moisture and fat content. A plant's moisture content is a key indicator of its safety and survival. *P. amarus* has a high moisture value, which means it will foster the growth of rotting microbes, lowering the plant's shelf life (Umofia et al. 2017). The presence of ash in the contents suggests that *P. amarus* is high in ash, making it a good source of plant minerals for man's normal metabolic activity and vitamin absorption (Umoh et al. 2013). It also contains a lot of fiber, which helps to prevent constipation, maintain proper digestive system function, and prevent colon cancer, as well as lower cholesterol and control blood sugar levels (Dhingra et al. 2012).

Taxonomy of *P. amarus:*

Kingdom - Plantae

Subkingdom - Tracheobionta

Class - Magnoliopsida

Superdivision -Spermatophyta

Order - Malpighiales

Family - Euphorbiaceae

Genus - *Phyllanthus* L.

Species - *Phyllanthus* niruri L.

Figure 2.1 *P. amarus* (Jenna et al. 2020).

2.1.1 Reported phytochemical constituents of *P. amarus*

Phytochemicals are bioactive plant molecules found in fruits, vegetables, grains, and other plant foods that have potential health benefits beyond basic nutrition, such as lowering the risk of major chronic diseases (Lui et al. 2003). Large levels of phenolic compounds, alkaloids, saponins, flavonoids, and terpenoids were found in aerial portions of the plant, such as the stem and leaf, by qualitative and quantitative analysis of numerous plant sections of P. amarus (Obuotor et al. 2007). Phyllanthus amarus' therapeutic value is derived from bioactive phytochemical elements that have a physiological effect on the human body (Obuotor et al. 2007). Alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds, and many more bioactive phytochemical elements are among the most important (Edeoga et al. 2005).

Q. amarus has been used to cure a variety of ailments in the past, including jaundice, kidney stones, fever, fertility concerns, and menstrual cycle problems (Ibukun et al, 2007). Antimalarial, anticancer, antioxidant, and other pharmacological properties have all been documented for the plant. Alkaloids have antibacterial function, phenolic compounds have antioxidant activity, flavonoids have antipyretic effect, and terpenoids have antipyretic activity (Ibukun et al, 2007). P.amarus contains a variety of chemical substances, including alkaloids, flavonoids, hydrolysable tannins (Ellagitannins), major lignans, polyphenols, triterpenes, sterols, and volatile oil. The plant's lignans included phyllanthin (a bitter ingredient) and hypophyllanthin (a bitter constituent) (a non bitter constituent)

The largest concentrations of phyllanthin (0.7 percent w/w) and hypophyllanthin (0.3 percent w/w) were detected in the leaves, while these compounds were found in minor amounts in the stem (Chevallier, 2000). (Edeoga et al. 2005).

2.2 Inflammation

At the tissue level, inflammation is characterized by redness, swelling, heat, discomfort, and a loss of tissue function. Local immune, vascular, and inflammatory cell responses to infection or damage cause it (Akira et al. 2010). These can result in DNA damage, tissue death, and internal scarring, all of which have been linked to the development of a number of diseases, including cancer (Akira et al. 2010). Because inflammation mediates and is the primary driver of many medical disorders and autoimmune diseases, such as ankylosing spondylitis, psoriasis, psoriatic arthritis, behcet's disease, arthritis, and inflammatory bowel disease, inflammation is an important and growing area of biomedical research and health care (Akira et al. 2010). When a physical cause activates an immunological response, inflammation occurs. Inflammation may not always indicate the presence of an infection, but infection can lead to inflammation. Inflammation plays a significant role in acute injuries as well as chronic disorders. Due to its extensive and widespread nature, inflammation is believed to have an impact on every aspect of normal human physiology and pathology (Medzhitoz, 2010).

2.2.1 Inflammatory response

When tissues are exposed to damaging inflammogens, the inflammatory response is a critical component of their responses. This complex process involves leukocytes, often known as inflammatory cells, such as macrophages, neutrophils, and lymphocytes (Halliwell et al. 2015). In response to the inflammatory process, these cells release specialized substances such as vasoactive amines and peptides, eicosanoids, proinflammatory cytokines, and acute-phase proteins that mediate the inflammatory process by preventing further tissue damage and eventually leading to healing and tissue function restoration (Halliwell et al. 2015).

Immunological and inflammatory responses are the body's primary defense system against a variety of externally injurious events and pathological events that arise from endogenous aberrant genetic and metabolic abnormalities (Miller et al. 2009). Immunological and inflammatory responses, on the other hand, can induce autoimmune disorders, granulomatous difficulties, cancer, respiratory, joint, and bone ailments, and

atherosclerosis. As a result, anti-inflammatory drugs are emerging as a promising source of new treatments for a variety of diseases, including cancer, metabolic syndrome, atherosclerosis, joint and bone diseases, and respiratory tract diseases (Miller et al. 2009).

Many pathological pain situations are exacerbated and prolonged by inflammatory reactions in the peripheral and central nervous systems. Certain inflammatory cytokines have been linked to pain behaviors and the development of aberrant spontaneous activity from injured nerve fibers or compressed/inflamed DRG neurons in the spinal cord, dorsal root ganglion (DRG), wounded nerve, and skin. Cytokines are small proteins released by cells that have a specific impact on cell interactions and communication (Miller et al. 2009).

Inflammatory cytokines are a type of cytokine (a signaling molecule) that is produced by immune cells and other cell types and causes inflammation. T helper cells and macrophages are the main producers of inflammatory cytokines, which play a role in the activation of inflammatory responses (Miller et al. 2009). Activated macrophages release proinflammatory cytokines, which are involved in the up-regulation of inflammatory processes. According to an increasing amount of evidence, pro-inflammatory cytokines including IL-1, IL-6, and TNF- are involved in the pathological pain process (Miller et al. 2009).

2.2.2 Types of inflammation

Acute inflammation: This is a short-term reaction to tissue injury that takes minutes or hours to manifest. Pain, redness, immobility (lack of function), swelling, and heat are the five cardinal indicators (Adam, 2020).

Fever, raised blood leukocyte counts, and the presence of acute phase proteins like fibrinogen and C-reactive protein in the plasma are all signs of an acute inflammatory response. If the acute inflammatory reaction includes veins, intravascular thrombosis can ensue, restricting venous blood flow (Adam, 2020). Exposure to bee stings or dust, as well as an injury or infection, can cause acute inflammation (Adam, 2020).

Chronic inflammation: This is also known as gradual, long-term inflammation because it lasts for a long time (Adam, 2020). Chronic inflammation has different impacts depending on the nature of the injury and the body's ability to repair and overcome the harm. When a person has sensitivity, exposure, autoimmune illnesses, auto-inflammatory diseases, or persistent acute inflammation, chronic inflammation can develop. Older age, obesity, smoking, stress, sleeping issues, and low sex hormones are all factors that might cause chronic inflammation (Adam, 2020).

Table 2.1 shows differences between acute and chronic inflammation

Table 2.1: Comparison between acute and chronic inflammation (Adam, 2020).

	Acute	Chronic
Cause	Harmful pathogens or tissue injury.	Pathogens that the body cannot breakdown, including some type of viruses, foreign bodies that remain in the system, or overactive immune responses.
Onset	Rapid.	Slow.
Duration	A few days.	From months to years.
Outcomes	Inflammation improves, or an abscess develops or becomes chronic.	Tissue death, thickening, and scarring of connective tissue.

2.2.3 Causes of inflammation

Exposure to chemicals such as bee stings or dust, as well as an injury or infection, can trigger acute inflammation (Adam, 2020).

An ingrown toenail, a sore throat from a cold or flu, physical trauma or a wound, and acute bronchitis or appendicitis are all examples of conditions or illnesses that can induce acute inflammation (Adam, 2020).

Chronic inflammation occurs when a person has:

- Sensitivity: When the body detects something it shouldn't, inflammation occurs. An allergy can develop as a result of hypersensitivity to an external stimulus.
- Chronic inflammation can be caused by long-term, low-level exposure to an irritant, such as an industrial chemical.
- Chronic acute inflammation: A person may not fully heal from acute inflammation in some instances. This can sometimes result in persistent inflammation.

Older age, obesity, sleep problems, low sex hormones, stress, and smoking are all risk factors for chronic inflammation (Adam, 2020).

2.2.4 Treatment of inflammation

Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat inflammation (Oguntibeju, 2018). Many nonsteroidal anti-inflammatory medicines (NSAIDs) are known to alleviate pain and inflammation by inhibiting arachidonic acid processing by isoforms

of the cyclooxygenase enzyme (COX-1 and/or COX-2), hence lowering prostaglandin synthesis (Oguntibeju, 2018). They are responsible for about 5% to 10% of all prescriptions written each year (Supakanya et al. 2018).

One of the most important therapeutic features of NSAIDs is their ability to decrease the production of particular prostaglandins (PGs) by inhibiting the cyclooxygenase enzymes (COX-1 and COX-2) (Supakanya et al. 2018). Examples of NSAIDs are diclofenac, ibuprofen and aspirin etc.

Inflammation can also be managed using pain relievers such as paracetamol, which do not lower inflammation but rather assist inflammation in continuing to play a part in healing (Adam, 2020). Corticosteroids, such as cortisol, which is a type of steroid hormone that affects several systems involved in inflammation, also aid in the treatment of inflammation (Adam, 2020).

2.2.5 Diclofenac potassium tablets USP, 50 MG

Potassium diclofenac tablet USP is a benzene acetic acid derivative, meaning it contains both benzene and acetic acid. They come in the form of orange film-coated capsules that can be taken orally. 2((2,6-dichlorophenyl) amino) benzene acetic acid, monopotassium salt is the chemical name. CHCIKNO is the structural formula, and the molecular weight is 334.24. Diclofenac potassium is a yellowish white to light beige crystalline powder with no odor and a slight hygroscopicity (Carilion material management, 2017).

Methanol, ethanol, and water are soluble, whereas chloroform and dilute acid are practically insoluble. The n-octanol/water partition coefficient is 13.4 at pH 7.4, but 1545 at pH 5.2. It has a single dissociation constant (pKa) of 4.0 0.2 at 25 degrees Celsius. Each tablet contains 50 milligrams of diclofenac potassium for oral use (Carilion material management, 2017).

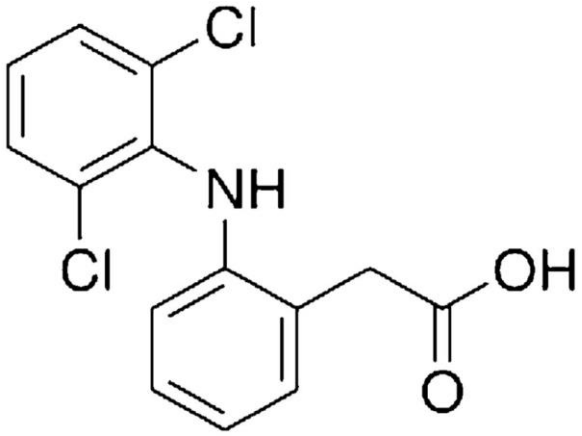

Figure 2.2 Chemical Structure of diclofenac (Carilion material management, 2017).

Mechanism of action

Analgesic, anti-inflammatory, and antipyretic effects are all present in diclofenac. Diclofenac potassium pills' mechanism of action, like that of other NSAIDs, is uncertain, although it involves suppression of cyclooxygenases (COX-1 and COX-2) (Carilion material management, 2017). Diclofenac significantly inhibits prostaglandin production this prostaglandins activate afferent neurons and enhance the role of bradykinin in pain generation in animal models. Diclofenac's mechanism of action is linked to a decrease in prostaglandin levels in peripheral tissues since prostaglandins are inflammatory mediators and diclofenac is a prostaglandin synthesis inhibitor (Carilion material management, 2017).

2.2.6 Mediators of inflammation

Inflammatory mediators are messengers that work on blood vessels and/or cells to generate an inflammatory response (Halliwell et al. 2015). These cells release specialized substances like vasoactive amines and peptides, eicosanoids, proinflammatory cytokines, and acute-phase proteins in response to the inflammatory process, which help to mediate the inflammatory process by preventing further tissue damage and eventually leading to healing and recovery. Chemical mediators from the circulatory system, inflammatory cells, and injured tissue all play a role in the inflammatory response and help regulate it (Halliwell et al. 2015).

This chemical mediators of inflammation are:

- Vasoactive amines such as histamine (Basophils release a few pictograms of histamine to maintain the acute-phase response during inflammatory events) and serotonin (Decarboxylation of tryptophan produces serotonin, which is then stored in the granule. Serotonin is found in basophilic granules in mice, but it is found in platelets in humans) (Gilfillan et al. 2011).
- Peptide such as bradykinin (Bradykinin is a nanopeptide produced by the Kinin–Kallikrein system in the blood. For bradykinins, two or more different receptors have been identified, dubbed B1 and B2. It can enhance prostaglandin synthesis and cause pain in the same way that histamine and serotonin do) (Weissmann, 2013).
- Eicosanoids: Arachidonic acid, which is the primary component of all cell membrane phospholipids, is one of the most essential substrates for the production of biologically active inflammatory mediators known as eicosanoids. E.g thromboxanes, leukotrienes, and prostaglandins (Mak et al 2013).

2.2.7 Markers of inflammation

Inflammatory markers consist of biomarkers used in clinical practice to determine whether a patient has an active inflammatory disease process.

- **C reactive protein (CRP)**: Inflammation causes the liver to create C-reactive protein (CRP). High-sensitivity C-reactive protein (hs-CRP) and ultra-sensitive C-reactive protein are two more names for it (us-CRP) (Robin, 2019). CRP is a blood marker of inflammation that can be caused by a wide range of disorders, from infection to cancer. High CRP levels can also indicate inflammation in the arteries of the heart, which can raise the risk of a heart attack. A CRP test can be used to diagnose inflammatory autoimmune illnesses like inflammatory bowel disease, lupus, and rheumatoid arthritis (Robin, 2019).
- **Erythrocyte sedimentation rate**: This is a test that evaluates inflammation in the body in a non-invasive manner. The test measures how quickly erythrocytes (red blood cells) settle (sediment) in a blood sample put into a tall, thin vertical tube. An exceptionally high ESR, especially in the absence of inflammation, can indicate the presence of malignant tumors (Bray et al. 2016). ESR test results that are higher than usual have also been connected to autoimmune diseases like lupus. Pregnancy, obesity, kidney illness, anemia, and thyroid disease all contribute to a high ESR. A low ESR is linked to disorders that hinder red blood cells from sedimenting appropriately, such as polycythemia (high red blood cell count), leukocytosis (high white blood cell count), and protein abnormalities. Congestive heart failure, low plasma protein (as a result of liver or kidney disease), and hypofibrinogenemia (too little fibrinogen in the blood) all contribute to a low ESR (Bray et al. 2016).
- **Plasma viscosity:** Plasma viscosity measurement can be used to determine the viscosity, or thickness, of the blood. The presence of proteins that can be produced as a normal response to infection or inflammation, or proteins that are produced improperly in some disorders, affects the viscosity of plasma (the liquid part of the blood) (paraproteins) (Bray et al. 2016).

2.3 Oxidative stress

Any disturbance in the equilibrium of antioxidants and pro-oxidants in favor of pro-oxidants, as a result of many variables such as age, inflammation, pharmacological action, and toxicity, is referred to as oxidative stress (Asmat et al. 2015). Oxidative stress causes various body cells' processes to be inefficient, as well as structural damage to cells,

resulting in a variety of diseased conditions (Asmat et al. 2015). Examples of structural impairment and diseased diseases produced by oxidative stress are listed in the table 2.2 below.

Table 2.2: Organs affected and diseases caused by oxidative stress (Asmat et al. 2015)

Organs	**Diseases**
Kidneys	Glomerulonephritis, chronic renal failure
Fetus	Preeclampsia, IU growth restriction
Eyes	Cataract. Retinal diseases
Brain	Alzheimer's disease, parkinson's disease, memory loss and stroke
Joints	Arthritis, rheumatism
Heart vessels	Arteriosclerosis, hypertension, cardiomyopathy, heart failure

Oxidative stress is caused by two different routes. It all starts with a decrease in antioxidant levels (which may be as a result of mutated antioxidants enzymes, toxins, or low intake of nutritional antioxidants). Second, as a result of ongoing inflammation, an increase in the quantity of free radicals, such as reactive oxygen species (ROS), causes oxidative stress (Somogyi et al. 2007). Though oxygen is required for life, it can induce necrosis and cell death when it oxidizes and creates reactive species. Exogenous reactive oxygen species include the hydroxyl radical, superoxide radical, and hydrogen peroxide (ROS). Cellular ROS include NADPH oxidase and hydroperoxyl radicals (Somogyi et al. 2007). Due to its two unpaired electrons with a parallel spin in different anti-bonding orbitals, oxygen possesses the feature of free radicals, which promotes its stability and paramagnetic property. This can be modified by a single electron transfer that changes oxygen to an oxidizing agent, and the enzyme oxidase catalyzes electron transfer to oxygen to produce energy or substrate oxidation (Chikezie et al. 2015).

Reactive oxygen species include both free radicals and non-radical oxygen species (ROS). Hydroxyl, superoxide, and nitric oxide are examples of free radicals, while hydrogen perioxide, ozone, and lipid perioxide are examples of non-radical reactive oxygen (Somogyi et al. 2007). Reactive oxygen species can be produced by the mitochondrial respiratory chain or by the activation of NADPH oxidases. If ROS become unwanted metabolic byproducts as a result of oxidative stress, this can lead to ROS overproduction,

which can alter proteins and DNA while also causing lipid perioxidation, causing cell damage or injury (Somogyi et al. 2007).

The three types of free radicals are reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS) (RCS). Because of their high reactivity, any of these, notably the ROS and RNS, which are more abundant in macromolecules and are involved in virtually all of the system's essential metabolisms, could produce oxidative stress. (Somogyi et al. 2007).

It is impossible to completely avoid free radical exposure and oxidative stress. Increasing antioxidant levels and lowering free radical production, on the other hand, can help to attenuate the effects of oxidative stress (Somogyi et al. 2007).

2.3.1 Oxidative stress and inflammation

Oxidative stress is defined as a discrepancy between the creation of reactive oxygen species (ROS) and their removal by protective systems, which can result in chronic inflammation. Oxidative stress-induced inflammation is the root of many chronic diseases. Chronic inflammation can be caused by oxidative stress. Infections and injuries activate the immune system. While fighting off invading bacteria, immune cells called macrophages release free radicals. These free radicals can cause inflammation by damaging healthy cells (Berlett et al. 1997).

Microbial and viral infections, allergen exposure, radiation, and toxic chemicals, autoimmune and chronic disorders, obesity, alcohol and cigarette use, and a high-calorie diet have all been linked to inflammation as a natural defensive mechanism against pathogens (Salzanoa et al. 2014). Many chronic disorders linked to increased ROS generation have oxidative stress and a variety of protein oxidation as a symptom. Inflammatory signaling molecules such as peroxiredoxin 2 are produced as a result of protein oxidation (Salzanoa et al. 2014).

Oxidative stress plays a negative role in chronic inflammatory diseases. Neuron degeneration is produced by oxidative stress, which includes oxidized proteins, glycated products, and lipid peroxidation. It is most typically found in brain illnesses (Mitran et al. 2013). The creation of reactive oxygen species (ROS) in brain tissues can disrupt synaptic and nonsynaptic transmission between neurons, causing neuroinflammation, cell death, and eventually dementia and memory loss (Mitran et al. 2013).

Glutathione tripeptide (GSH) is an intracellular thiol antioxidant; a low level of GSH leads to an imbalanced immune response, inflammation, and infection susceptibility (Ghezzi,

2011). Inflammatory stimuli cause peroxiredoxin 2 (PRDX2), a ubiquitous redox-active intracellular enzyme, to be released. After being released, it acts as a redox-dependent inflammatory mediator, encouraging macrophages to produce and release TNF. Glutathionylation of the PRDX2 protein by oxidative linked GSH occurs before or during the release of PRDX2, and this affects immunity (Salzano et al. 2014).

PRDX2 is also a part of the inflammatory cascade, and it has the power to cause TNF- to be released. Despite the fact that PRDX2 is constantly present in macrophages but at a lower level when stimulated by LPS and then released in an oxidized form, it has no effect on mRNA or protein synthesis mediated by liposaccharide (LPS), despite the fact that it is constantly present in macrophages but at a lower level when stimulated by LPS and then released in an oxidized form (Salzanoa et al. 2014). This research discovered that macrophage-derived PRDX2 and thioredoxin (TRX) can alter the redox status of cell surface receptors, allowing for the activation of an inflammatory response, pointing to a novel therapeutic target for chronic inflammatory illnesses (Salzanoa et al. 2014). Chronic inflammation is involved in the pathogenesis of several diseases such as insulin resistance, type 2 diabetes mellitus (T2DM), and cardiovascular diseases (CVD), obesity related chronic inflammation factors (Khan, 2012) are described in Figure 2.3

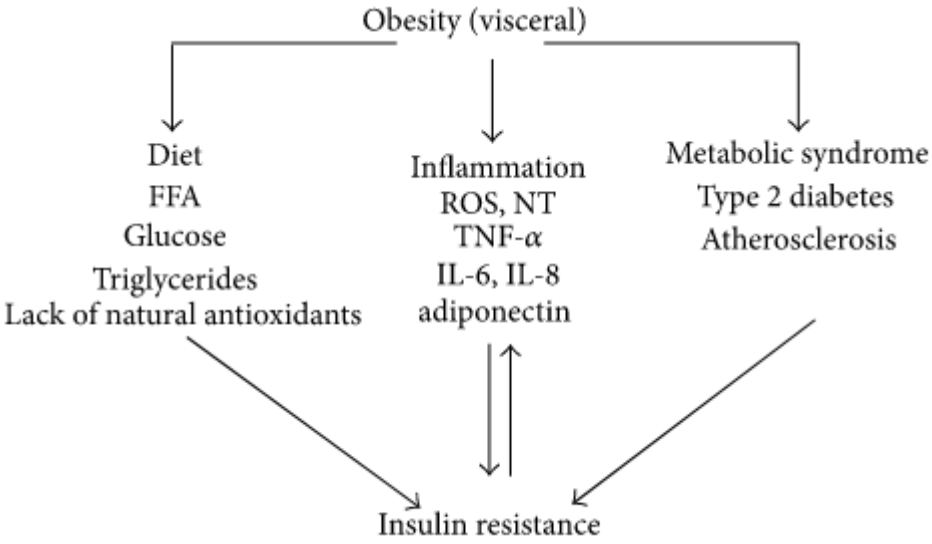

Figure 2.3 : Obesity lifestyle development of chronic diseases through inflammation (Micol et al. 2015).

2.4 Anti-inflammatory agents

The property of a medication or treatment that reduces inflammation or swelling is known as anti-inflammatory. Anti-inflammatory medications account for nearly half of analgesics, and they function by lowering inflammation rather than opioids, which block pain signals to the brain through acting on the central nervous system. Aspirin is one example of an anti-inflammatory (Ghasemian et al. 2015).

Inflammation is a comprehensive pathologic condition that includes rheumatoid and immune-mediated illnesses, diabetes, and cardiovascular disease (Ghasemian et al. 2015).

2.4.1 Lipid profile

A lipid profile, often known as a lipid panel, is a set of blood tests used to detect abnormalities in lipids such as cholesterol and triglycerides. A lipid profile includes the levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, the calculated low-density lipoprotein (LDL) 'cholesterol and the very low-density lipoprotein (VLDL) cholesterol (Niva et al. 2019).

2.4.1.1 Cholesterol

Cholesterol is an unsaturated alcohol from the steroid family that is essential for the normal function of all animal cells and is a component of their cell membranes. It's also a precursor for essential molecules including steroid hormones from the adrenal and gonadal glands, as well as bile acids. Cholesterol is a crucial component of the human body's lipid fraction.(Niva et al. 2019). Cholesterol must be carried in the plasma in association with various lipoprotein particles because it is a nonpolar lipid molecule (insoluble in water). The hydration density, electrophretic mobility, and size of plasma lipoproteins are used to classify them (Niva et al. 2019).

2.4.1.2 Triglyceride

Triglycerides are lipid molecules made up of three fatty acid chains of varying lengths and compositions esterified to glycerol. The chemical composition of these fatty acid chains varies, and they can be saturated or unsaturated. Depending on the degree of saturation or unsaturation, each chain is made up of carbon and hydrogen atoms with various single or double-bonded chains. A substantial amount of triglyceride can be found in the lipid portion of the human body. Triglycerides (Niva et al. 2019) are fatty acid esters of glycerol that make up the majority of dietary fat and animal fat storage. Triglyceride, being a nonpolar lipid substance (insoluble in water), need to be transported in the plasma associated with various lipoprotein particles. Plasma lipoproteins are separated by hydrated density, electrophretic mobility, and size (Niva et al. 2019).

2.4.1.3 HDL-cholesterol

High-density lipoprotein (HDL) cholesterol is known as the "good" cholesterol because it helps remove other forms of cholesterol from the bloodstream. Higher levels of HDL cholesterol helps to lower the risk of heart disease (Niva et al. 2019).

2.4.1.4 LDL-cholesterol

In the bloodstream, LDL cholesterol is a form of lipoprotein (a fat-protein combination). Because it takes cholesterol from the bloodstream and delivers it to the cells, as well as producing cholesterol buildup in the arteries, it is referred to as "bad" cholesterol. As LDL cholesterol levels rise, the risk of heart and blood vessel disease rises as well (Niva et al. 2019).

2.4.1.5 VLDL-cholesterol

VLDL cholesterol (very low-density lipoprotein) is a type of lipid present in the blood. It is one of the "bad" kinds of cholesterol, along with LDL cholesterol and triglycerides, because high cholesterol levels can clog arteries and cause heart attacks. (VLDL) cholesterol is produced in the liver and subsequently transferred into the bloodstream to offer a type of fat (triglycerides) to biological tissues (Niva et al. 2019).

2.5 Antioxidants

Antioxidants are chemicals that prevent a substrate from oxidizing (Somogyi et al. 2007). Antioxidants are compounds that can help prevent or postpone cell damage caused by free radicals, which are unstable molecules that the body produces in reaction to various stimuli. "Free-radical scavengers" is another name for them. They are available in both natural and synthetic forms (Somogyi et al. 2007).

Antioxidants are classified as either breakdown antioxidants or preventive antioxidants, depending on how they work (Somogyi et al. 2007). Catalase and peroxidase are examples of preventive antioxidants that react with ROOH and metal chelators such as ethylene diamine tetra acetate (EDTA). This class of antioxidants works by preventing the uncontrolled synthesis or production of free radicals and inhibiting their reactions with biomolecules; an example is how superoxide dismutase (SOD) traps superoxide radicals in vivo (Somogyi et al. 2007).

Antioxidants are important in the body's defense against reactive oxygen species (ROS) (Boxin et al. 2002). According to the Food and Drug Administration, antioxidants are dietary supplements that are taken in addition to regular food intake to help prevent diseases (Ohlsson et al. 2002). Antioxidants that come from outside sources are known as exogenous antioxidants. Antioxidants are generally obtained through diet, however

because exogenous antioxidants are very impossible to come by in modern diets, antioxidant supplementation is advised. Exogenous antioxidants include vitamins A, C, and E; however, endogenous antioxidants include glutathione, superoxide dismutase, and catalase (Asmat et al. 2015).

2.5.1 Lipid peroxidation

Different aldehydes, ketones, alkanes, carboxylic acids, and polymerization products are generated when free radicals (such as OH, O2–, and H2O2) attack phospholipids or PUFA in the membranes of cellular or subcellular components (Rahul et al. 2020). These compounds are lipid peroxidation indicators because they react strongly with other cellular components and the extracellular matrix (Rahul et al. 2020). Malondialdehyde (MDA) is a toxic aldehydic end product of lipid peroxidation that accelerates cartilage collagen oxidation, resulting in fragmentation, alteration, aggregation, and structural changes in proteins, ultimately leading to tissue failure (Rahul et al. 2020).

2.5.2 Superoxide dismutase activity

SOD is an enzyme that catalyzes the conversion of superoxide to oxygen and hydrogen peroxide, regulates the amount of reactive nitrogen and oxygen species in the body, and controls the autoxidation of adrenaline (Wang et al. 2018). Superoxide dismutase is an antioxidant-protective signaling molecule that modulates signals and regulates RNS and ROS levels by interacting with superoxide. When superoxide dismutase activity is enhanced, the risk of lipid peroxidation is lowered (Wang et al. 2018). The reaction of superoxide dismutase makes it a good assay marker; the superoxide radical is formed by the reaction of xanthine oxidase, that may contribute to the development of adenophrine from the oxidation of epinephrine, that raises the pH and the concentration of epinephrine at the same time (Wang et al. 2018).

2.5.3 Catalase activity

One of the most important antioxidant enzymes is catalase. Almost every aerobic organism contains it. In a two-step reaction, catalase breaks down two hydrogen peroxide molecules into one molecule of oxygen and two molecules of water (Hausner et al. 1993). The chemical reaction begins with the reduction of one hydrogen peroxide molecule, yielding a spectroscopically unique intermediate product, a covalent oxyferryl species (FeIVO) with a porphyrin -cation radical (Ivancich, et al. 1997).

The free enzyme, oxygen, and water are produced in the second step of the process by a two-electron transfer from an electron donor (the second molecule of hydrogen peroxide) via redox reactions (Deisseroth et al. 1970).

2.5.4 Reduced level of glutathione concentration

Reduced glutathione is a homodimer that contains one FAD per monomer and catalyzes the reduction of glutathione disulfide (GSSG) to glutathione (GSH) (Rebecca et al. 2010). Reduced glutathione replaces intracellular GSH via lowering GSSG in the presence of NADPH and flavine adenine dinucleotide (FAD), a derivative of the water-soluble vitamin riboflavin. GSSG and GSH are important components of cellular redox balance. GSH provides reducing equivalents to glutathione peroxidase, which helps it detoxify reactive oxygen species, and glutathione S-transferase detoxifies electrophilic xenobiotics. Glutathione reductase is one of several enzymes that maintain glutathione's reduced form (Rebecca et al. 2010).

The NADPH-catalyzed reduction of GSSG to GSH is catalyzed by glutathione reductase. The widespread belief that disulfide buildup is detrimental for cells drove the widespread adoption of theories that thiol S-thiolation events are essential for oxidant-mediated cell death (Xylina et al. 2018).

CHAPTER THREE
METHODOLOGY

3.1 Materials and Reagents

The materials and equipment used in this study include:

Volumetric flask, weighing balance, filter paper, funnel, dropper, beaker, rotary evaporator, spatula, nose mask, hand gloves, sample bottle rack, petri dishes, alluminium foil, sample bottles, eppendorf tubes, filter paper, jute bag, separating funnel, retort stand, distilled water.

The following reagents were used in this study: Ethanol, butanol, ethyl acetate, hexane, 0.9% normal saline, phosphate buffer saline, 3.2% tri-sodim citrate.

The following kits were used in this study: Cholesterol kit, HDL-cholesterol kit, Triglyceride kit, Catalase kit, Superoxide dismutase kit.

3.2 Methods

3.2.1 Authentication of experimental plant

The plant *Phyllanthus amarus* was identified in the Herbarium of the department of Botany University of Lagos with the voucher number 8786, and a sample deposited.

3.2.2 Collection and preparation of plant materials

P. amarus was were obtained within the premises of Mountain Top University in March 2021. The roots of the weed plant were removed and the leaves of the plant was dried in the hot air oven at 40 ^{o}C. The dried aerial parts were pulverized using a blender into a fine powder. The powdered *P. amarus* was stored in airtight container then kept in a refrigerator at 4 ^{o}C until further use. Eighty grams (80 g) of *P. amarus* were weighed into an airtight jar and immersed in a ratio of 70 % ethanol to 30 % water (8:1 w/v) for 72 h with intermittent shaking.

A sterile muslin cloth was used to filter the ethanol and plant sample mixture over an empty beaker. The filtrate was then concentrated in a rotary evaporator under reduced pressure at 40 ^{o}C. The percentage yield of the extract was calculated.

3.3 Experimental animals

Mice were weighed. Every morning upon entering the animal house, the mice were exposed to light for 12 hours and darkness for another 12 hours daily and they were given water and fed with ad libitum.

A total number of 45 male and female mice were obtained from the animal facility of Mountain Top University and acclimatized for seven days and were exposed to 12 hours light and dark cycle, respectively, and fed with standard chow and water *ad libitum*. The expermimental animals were randomly divided into 9 groups with each group containing 5 mice and they were all induced with inflammation with carrageenan except the normal control. The description of the groups are as follows:

Group 1 - Normal control

Group 2 - Negative control (carrageenan only)

Group 3 - Inflammed mice administered with standard drug (diclofenac)

Group 4 - Inflammed mice administered with 100 mg/kg of ethanol extract

Group 5 - Inflammed mice administered with 200 mg/kg of ethanol extract

Group 6 - Inflammaed mice administered with 100 mg/kg of ethyl acetate extract

Group 7 - Inflammed mice administered with 200 mg/kg of ethyl acetate extract

Group 8 - Inflammaed mice administered with 100 mg/kg of aqueous extract

Group 9 - Inflammed mice administered with 200 mg/kg of aqueous extract

3.3.1 Induction of inflammation

Inflammation was induced using carrageenan. Administration of plant extracts and diclofenac (standard treatment) started after 24 hours of induction of inflammation. Inflammation in mice was comfirmed by physical and biochemical measurement. The paw oedema was measured using a vernier caliper. The animals were treated with test samples for 12 days.

3.3.2 Sample collection Preparation of serum and organ samples

The experimental animals were sacrificed under anesthesia after seven days and the blood samples were collected by ocular puncturing. The blood sample (serum) was collected into plain bottles and centrifuge at 2500 g for 15 minutes which was supernatant was separated using pipette and stored in the refrigerator at 4 ℃. Organs (liver and kidney) were collected from the mice and homogenized with mortal and pestel, then centrifuged at 12000 g for 10 minutes, whereby they were separated from the pellet and stored in the refrigerator at 4 ℃.

3.4 Biological Assays

3.4.1 Assay for Cholesterol

The cholesterol assay was performed on the liver and serum using a Randox kit.

Along with one tube for blank and another for standard, tubes were labeled according to the experimental animal's identity and group. 10 L of the samples were pipetted into clean test tubes, 1000 L of the working reagent was added to each test tube, 10 L of the standard reagent was pipetted into a clean test tube, 1000 L of the working reagent was added to the test tube, 10 L of distilled water was pipetted into a clean test tube, 1000 L of the working reagent was added to the test tube, 10 L of distilled water was pipetted into a clean test tube, 1000 All of the tubes were incubated for 5 minutes at 37 °C, and the absorbances were measured using a spectrophotometer at 500 nm against a blank.The liver and serum were used for the cholesterol assay using Randox kit (Cole-Palmer Ltd, UK) . Concentration of cholesterol was obtained using the formula:

(Absorbance of sample/Absorbance of standard) x concentration of standard (203 mg/dL).

3.4.2 Assay for Triglyceride

The Meril kit was used to perform the triglyceride assay on the liver and serum.

Along with one tube for blank and another for standard, tubes were labeled according to the experimental animal's identity and group. 10 L of the samples were pipetted into clean test tubes, 1000 L of the working reagent was added to each test tube, 10 L of the standard reagent was pipetted into a clean test tube, 1000 L of the working reagent was added to the test tube, 10 L of distilled water was pipetted into a clean test tube, 1000 L of the working reagent was added to the test tube, 10 L of distilled water was pipetted into a clean test tube, 1000 The absorbances were measured at 500 nm against a blank after 5 minutes of incubation at 37 °C. The concentration of cholesterol was obtained using the formula:

(Absorbance of sample/Absorbance of standard) x concentration of standard (192 mg/dL).

3.4.3 Assay for HDL-cholesterol

The HDL-cholesterol was measured with a Randox kit utilizing the liver and serum.

Tubes were labeled according to the experimental animal's identification and group, and 200 liters of samples were pipetted into sample bottles, followed by 500 liters of reagent. The sample bottles were allowed to sit at room temperature for 10 minutes before being centrifuged at 4000 rpm for 10 minutes. Following centrifugation, the clear supernatant was pipetted from the sample bottles and placed in clean test tubes, together with a blank and a standard tube.10µL of the samples was pipetted into clean test tubes, 1000 µL of the working reagent was added to each test tubes, 10 µL of the standard reagent was pipetted into a clean test tube, 1000 µL of the working reagent was added to the test tube, 10 µL of

distilled water was pipetted into a clean test tube, 1000 µL of the working reagent was added to the test tube. All the tubes were incubated at 37 ℃ for 5 mins and the absorbances were read at 500 nm against blank. The concentration of cholesterol was obtained using the formula:

(Absorbance of sample/Absorbance of standard) x concentration of standard (203 mg/dL).

3.4.4 Estimation of LDL-cholesterol

This test was calculated using results from Cholesterol, HDL-cholesterol and VLDL-cholesterol:

Concentration of LDL-cholesterol (mg/dL) = Total chol - VLDL-chol - HDL chol

3.4.5 Estimation of VLDL-cholesterol

This test was calculated using result from triglyceride:

Concentration of VLDL-cholesterol (mg/dL) = Triglyceride conc./5

3.5 Assay for Lipid perioxidation

The amount of thiobarbituric acid reactive substances (TBARS) contained in the sample was used to determine the level of oxidative damage on the tissues. Malondialdehyde (MDA), which is formed by the acidic peroxidation of membrane fatty acids and food products, combines with the chromogenic reagent 2-thiobarbituric acid (TBA) to produce a pink-colored complex with a maximum absorbance of 532 nm.

Reagents used: Trichloroacetic acid (TCA, 30 %) (9 g of TCA was dissolved in distilled water and made up to 30 mL with same), Thiobarbituric acid (TBA, 0.75 %) (0.225 g of TBA was dissolved in 0.1M HCl and made up to 30 mL with same and Tris-KCl buffer (0.15 M, pH 7.4) (1.12g of KCl and 2.36 g of Tris base were

dissolved separately in distilled water and made up to 100 mL with same and pH was then adjusted to 7.4.

0.4 mL of the sample was pipetted into a clean test tube, 1.6mL of Tris-KCL buffer was added then 0.5 mL of 30% TCA. Then 0.5 mL of 0.75 % TBA was added and placed in a water bath for 45 minutes at 80^{o}C. After this, the samples were placed on the slab for it to get cool and it was separated using a centrifuge at 3000 g. The clear supernatant was pipetted from the sample bottle and the absorbance was measured against a reference blank of distilled water at 532 nm. The level of MDA was determined in units/mg protein with a molar extinction coefficient of 1.56 x 105 $M^{-1}cm^{-1}$.

3.6 Determination of Superoxide dismutase activity

An increase in absorbance at 480 nm was used to test the ability of superoxide dismutase to prevent the auto-oxidation of SOD reagent. The reaction mixture also contained 2.95 mL 0.05 M sodium carbonate buffer pH 10.2 (made by dissolving NaCo3.10H2O (14.3 g) and 4.2 g of NaHCO3 in 900 mL distilled water and correcting the pH to 10.2), 0.02 mL liver homogenate, and 0.03 mL 2 mM SOD reagent (used to activate the reaction) (3 mL). To make a blank, 2.95 mL sodium carbonate buffer and 0.03 mL SOD reagent were mixed together. For 5 minutes, the absorbance was measured at 480 nm at 1 minute intervals.

$\varepsilon = 4020 M^{-1} cm^{-1}$ (Zou et al., 1986) ,The activity of SOD = $\Delta A/min \times TV/\varepsilon \times SV$

Where: ΔA = change in absorbance, TV = total volume, SV = sample volume, ε = molar extinction

3.7 Determination of Catalase activity

1.0 mL of 0.01 M phosphate buffer (pH 7.0), 0.1 mL of liver homogenate, and 0.4 mL of 0.02 M H2O2 made up the reaction mixture (1.5 mL). The addition of 2.0 mL catalase reagent stopped the process. The absorbance was measured at 620 nm at 1 minute intervals against a reagent blank.

$\varepsilon = 40 M^{-1} cm^{-1}$, Catalase activity = $\Delta A/min \times TV/\varepsilon \times SV$

Where: ΔA = change in absorbance, TV = total volume, SV = sample volume, ε = molar extinction

3.8 Determination of reduced level of Glutathione concentration

The non-protein sulphybydryls glutathione (GSH) concentration of liver tissue and kidney was calculated. 1 mL of 10% TCA will be added to the sample and centrifuged at 3000 rpm. 0.5 mL Ellmans reagent and 3.0 mL phosphate buffer will be added to 5 mL of the supernatant (0.2 M, pH 8.0). At 412 nm, the absorbance will be measured against a reagent blank.

$\varepsilon = 1.34 \times 10^4 M^{-1} cm^{-1}$, The concentration of GSH = $\Delta A \times TV/\varepsilon \times SV$

Where : ΔA = change in absorbance, TV = total volume, SV = sample volume, ε = molar extinction

3.9 Statistical Analysis

The statistical analysis was carried out using Graph Pad Prism Software (GPPS 9.0). The results were reported as mean ± SEM (standard error of mean). The data collected were subjected to one way analysis of variance (ANOVA). Test of significance was at 0.05 % probability ($p < 0.05$).

3.10 Waste disposal

Experimental wastes were incinerated, and the mice carcasses were buried in designated location.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Percentage yield

Percentage yield = $\frac{\text{Weight after extract}}{}$ X 100

Weight before extract

The ethanol extract gave a yield of 62.25 %; while the aqueous and ethylacetate fraction yielded 33.58 % respectively.

4.2 Effects of Oral administration of aqueous, ethyl acetate and ethanol extract of *P. amarus* on the paw oedema of carrageenan induced inflammation in the mice

Figure 4.1, shows a significant ($p< 0.05$) decrease in the paw sizes within the first four hours after induction with carrageenan upon administration of various fractions of *P. amarus* extracts when compared to the control.

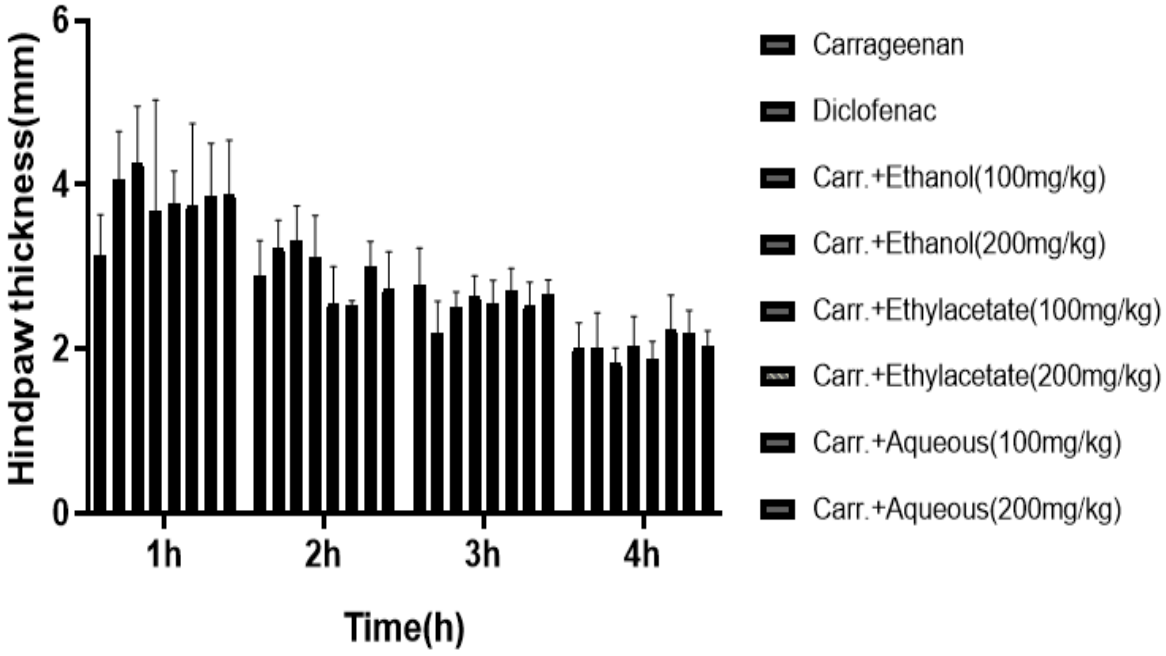

Figure 4.1: Carrageenan induced oedema of mouse pore.

Data are mean ± SD of replicate determination; n=4

Carr: Carrageenan

4.3 Effect of *P.amarus* ethanol extract on lipid profile in carrageenan induced inflammation in mice

The effect of *P.amarus* ethanol extract on cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol are shown in the figures 4.2 - 4.6.

4.3.1 Effect of *P.amarus* ethanol extract and fractions on serum and liver cholesterol levels of carrageenan induced inflammation in mice

Figure 4.2 shows that mice given the standard treatment had a significant increase ($p< 0.05$) in liver cholesterol levels when compared to the normal control, but the negative control had a higher cholesterol level than the normal control. When compared to the negative control, there were substantial decreases ($p<0.05$) in liver cholesterol levels after administration of all plant extracts.

In addition, as shown in figure 4.2, serum cholesterol levels in mice given the standard treatment decreased significantly ($p< 0.05$) when compared to the normal control, although the negative control had a little lower cholesterol level than the normal control. When compared to the negative control, blood cholesterol levels decreased significantly ($p< 0.05$) after administration of all plant extracts.

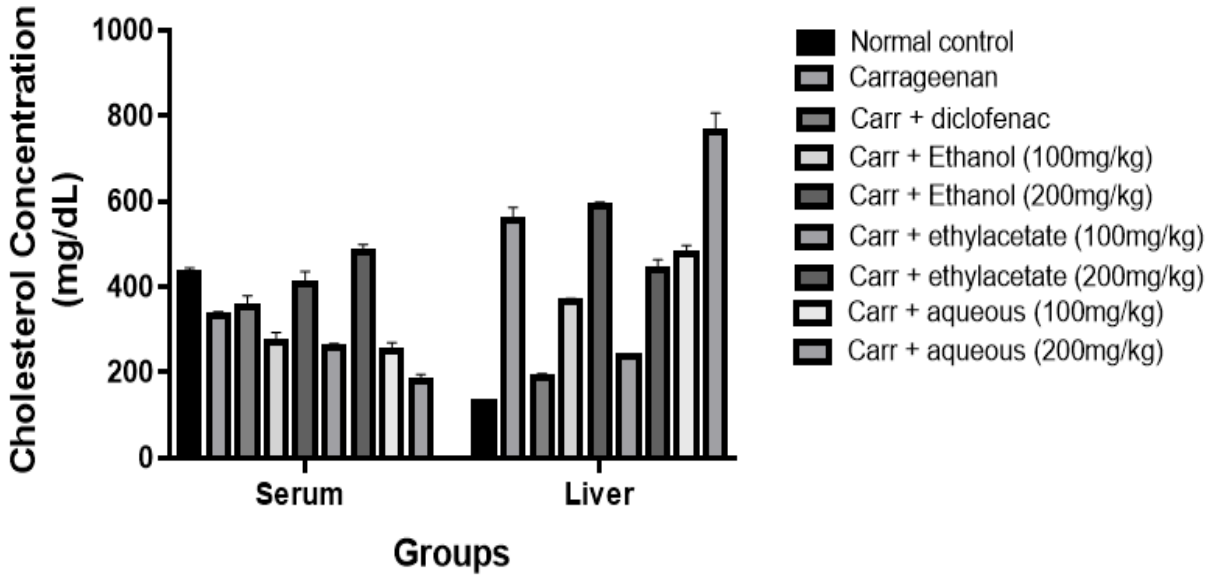

Figure 4.2 : Cholesterol levels of control and experimental mice with graded concentration of ethanol extract and fractions of *P. amarus*

Data are mean ± SD of replicate determination; n=4

Carr: Carrageenan

4.3.2 Effect of *P.amarus* ethanol extract and fractions on serum and liver triglyceride levels in carrageenan induced inflammation in mice

Figure 4.3 shows that mice given the standard treatment had a substantial rise ($p< 0.05$) in liver triglyceride levels when compared to the normal control, whereas the negative

control had a slightly higher triglyceride level than the normal control. When compared to the negative control, there were significant increases ($p < 0.05$) in liver triglyceride levels after administration of all plant extract.

In addition, as shown in figure 4.3, blood triglyceride levels in mice given the standard medication increased significantly ($p<0.05$) when compared to the normal control, whereas the negative control had a lower triglyceride level than the normal control. When compared to the negative control, there were significant increases ($p<0.05$) in serum triglyceride levels after administration of all plant extracts.

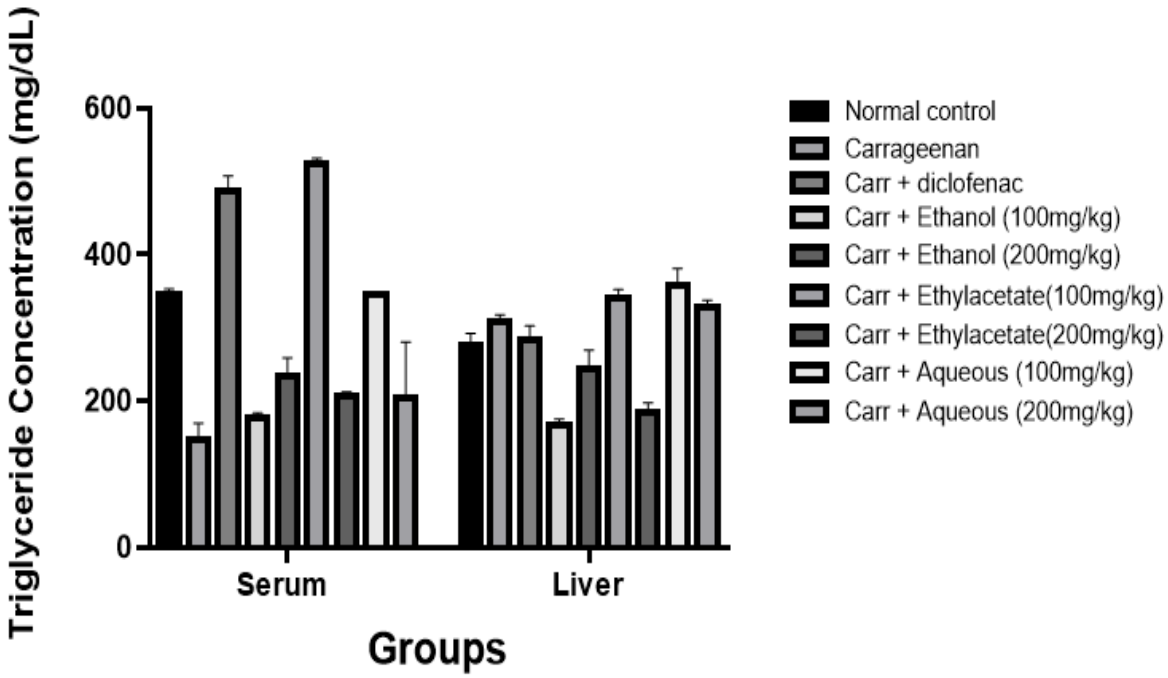

Figure 4.3 : Triglyceride levels of control and experimental mice with graded concentration of ethanol extract and fractions of *P. amarus*

Data are mean ± SD of replicate determination; n=4

Carr: Carrageenan

4.3.3 Effect of *P.amarus* ethanol extract and fractions on serum and liver HDL-cholesterol levels in carrageenan induced inflammation in mice

According to figure 4.4, mice given the standard treatment had a significant increase ($p<0.05$) in liver HDL-cholesterol levels when compared to the normal control, but the negative control had a higher HDL-cholesterol level than the normal control. When

compared to the negative control, there were substantial increases ($p<0.05$) in liver HDL-cholesterol levels after administration of all plant extracts.

In addition, as shown in figure 4.4, blood HDL-cholesterol levels in mice given the standard treatment increased significantly ($p< 0.05$) when compared to the normal control, although the negative control had a greater HDL-cholesterol level than the normal control. However upon administration of all plant extract there were significant increases ($p < 0.05$) in serum HDL-cholesterol levels when compared to negative control.

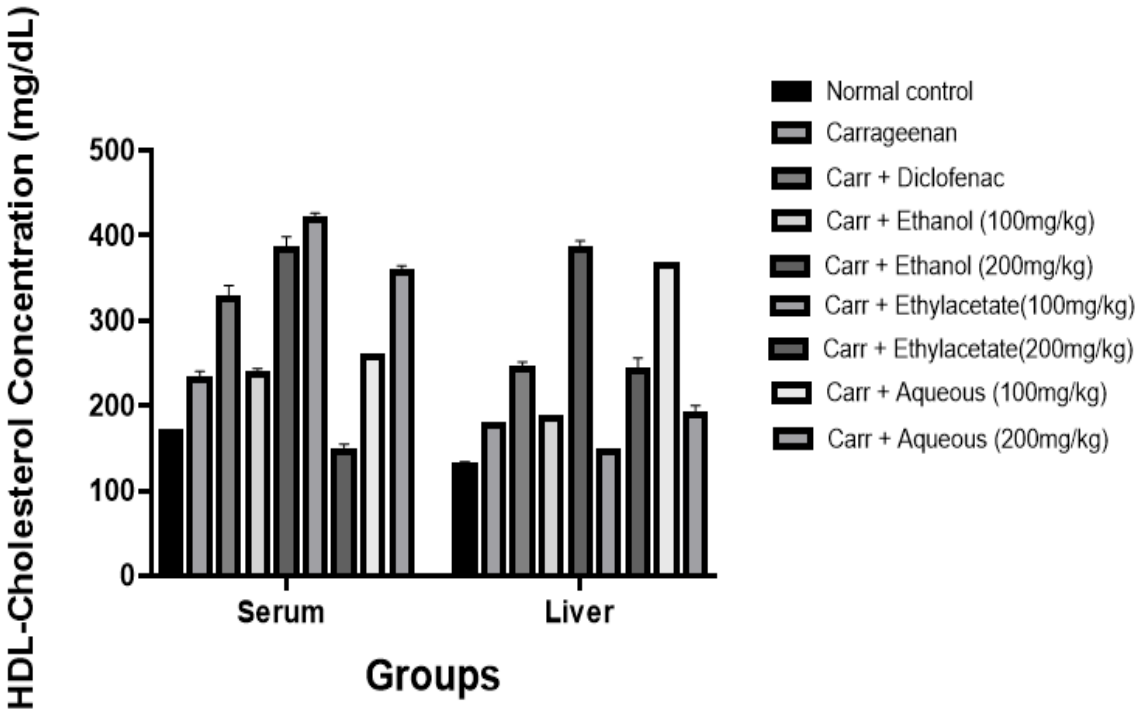

Figure 4.4 : HDL-cholesterol levels of control and experimental mice with graded concentration of ethanol extract and fractions of *P. amarus*

Data are mean ± SD of replicate determination; n=4

Carr: Carrageenan

4.3.4 Effect of *P.amarus* ethanol extract and fractions on serum and liver LDL-cholesterol levels in carrageenan induced inflammation in mice

According to figure 4.5, liver LDL-cholesterol levels in mice given the standard treatment decreased significantly ($p<0.05$) when compared to the normal control, whereas the negative control had a greater LDL-cholesterol level than the normal control. When

compared to the negative control, there were substantial decreases ($p<0.05$) in liver LDL-cholesterol levels after administration of all plant extracts.

In addition, as shown in figure 4.5, serum LDL-cholesterol levels in mice given the standard medication increased significantly ($p<0.05$) when compared to the normal control, but the negative control had a lower LDL-cholesterol level than the normal control. However upon administration of all plant extract there were significant increases ($p < 0.05$) in serum LDL-cholesterol levels when compared to negative control.

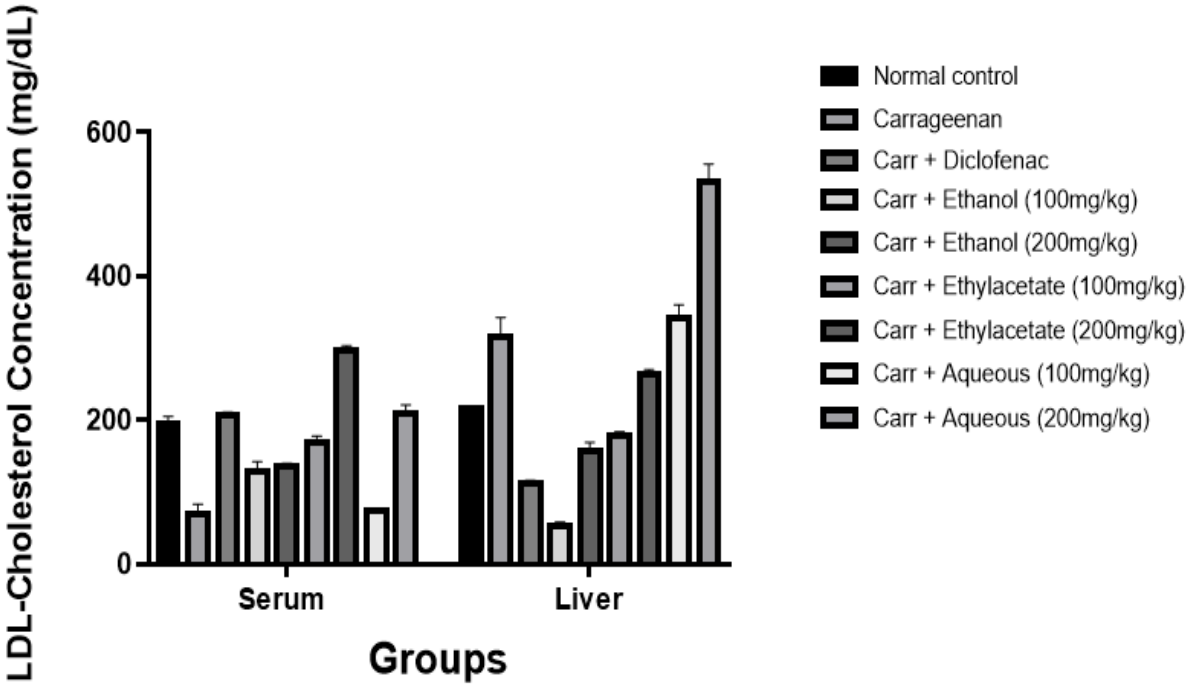

Figure 4.5: LDL-cholesterol levels of control and experimental mice with graded concentration of ethanol extract and fractions of *P. amarus*

Data are mean ± SD of replicate determination; n=4

Carr: Carrageenan

4.3.5 Effect of *P.amarus* ethanol extract and fractions on serum and liver VLDL-cholesterol levels in carrageenan induced inflammation in mice

From figure 4.6, there was a significant decrease ($p < 0.05$) of liver VLDL-cholesterol levels in mice administered with standard drug when compared to normal control, while the negative control recorded a lower VLDL-cholesterol level compared with the normal

control. However upon administration of all plant extract there were significant increases ($p < 0.05$) in liver VLDL-cholesterol levels when compared to negative control.

Also, from figure 4.6, there was a significant increase ($p < 0.05$) of serum VLDL-cholesterol levels in mice administered with standard drug when compared to normal control, while the negative control recorded a lower VLDL-cholesterol level compared with the normal control. However upon administration of all plant extract there were significant increases ($p < 0.05$) in serum VLDL-cholesterol levels when compared to negative control.

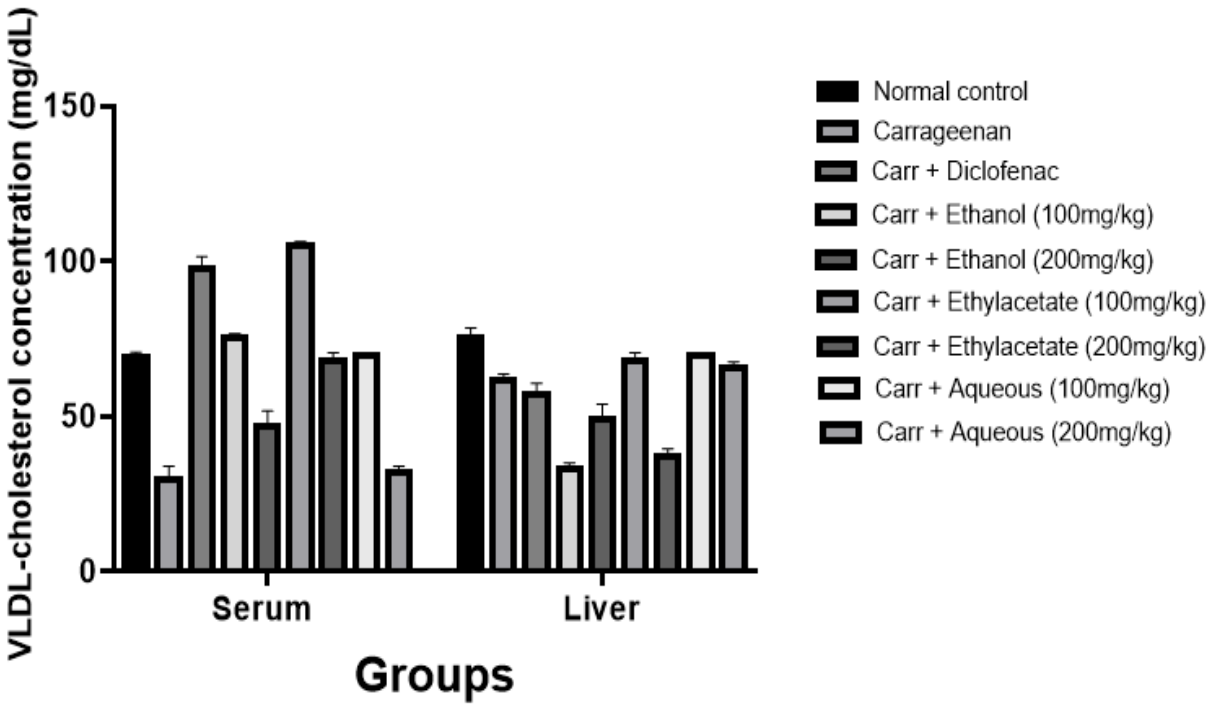

Figure 4.6: VLDL-cholesterol levels of control and experimental mice with graded concentration of ethanol extract and fractions of *P. amarus*

Data are mean ± SD of replicate determination; n=4

Carr: Carrageenan

4.4 Effect of *P.amarus* ethanol extract and fractions on the extent of kidney and liver lipid peroxidation in carrageenan induced inflammation in mice

From figure 4.7, there was a significant decrease ($p < 0.05$) of liver MDA concentration in mice administered with standard drug when compared to normal control, while the negative control recorded a lower MDA concentration compared with the normal control.

However upon administration of all plant extract there were significant decreases ($p < 0.05$) in liver MDA concentration when compared to negative control.

Also, from figure 4.7, there was a significant increase ($p < 0.05$) of kidney MDA concentration in mice administered with standard drug when compared to normal control, while the negative control recorded a higher MDA concentration compared with the normal control. However upon administration of all plant extract there were significant decreases ($p < 0.05$) in kidney MDA concentration when compared to negative control.

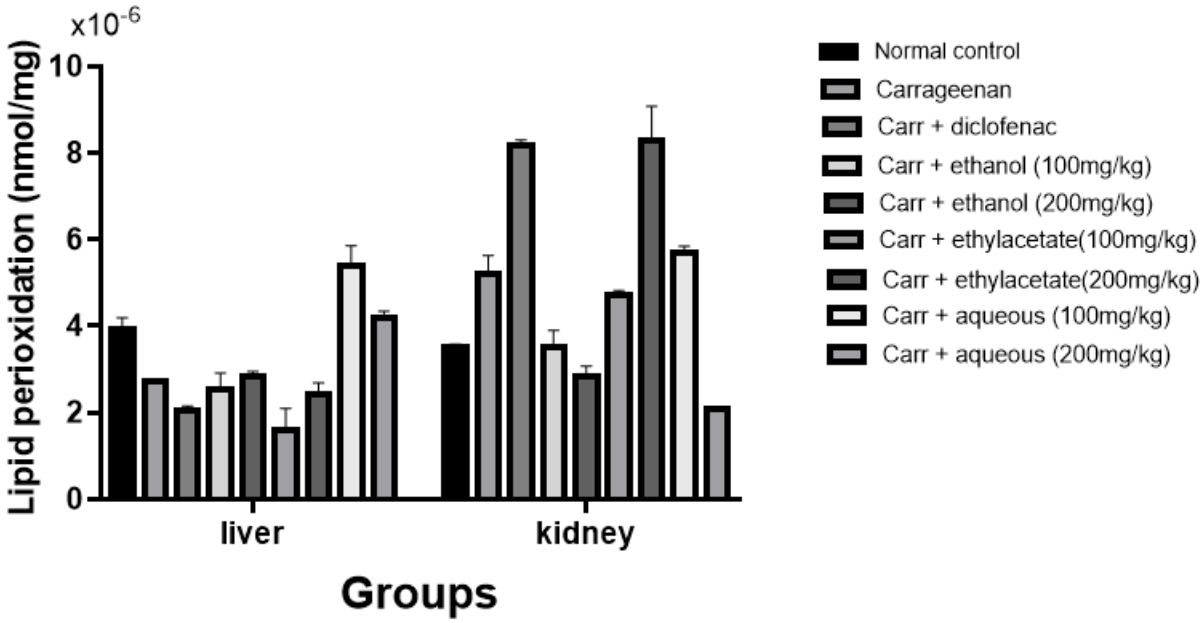

Figure 4.7: Lipid peroxidation concentration of control and experimental mice with graded concentration of ethanol extract and fractions of *P. amarus*

Data are mean ± SD of replicate determination; n=4

Carr: Carrageenan

4.5 Effect of *P.amarus* ethanol extract and fractions on kidney and liver on the Superoxide dismutase activity in carrageenan induced inflammatory mice

From figure 4.8, there was a significant decrease ($p < 0.05$) of liver SOD activity in mice administered with standard drug when compared to normal control, while the negative control recorded a lower SOD activity compared with the normal control. However upon

administration of all plant extract there were significant increases ($p < 0.05$) in liver SOD activity when compared to negative control.

Also, from figure 4.8, there was a significant decrease ($p < 0.05$) of kidney SOD activity in mice administered with standard drug when compared to normal control, while the negative control recorded a lower SOD activity compared with the normal control. However upon administration of all plant extract there were significant increases ($p < 0.05$) in kidney SOD activity when compared to negative control.

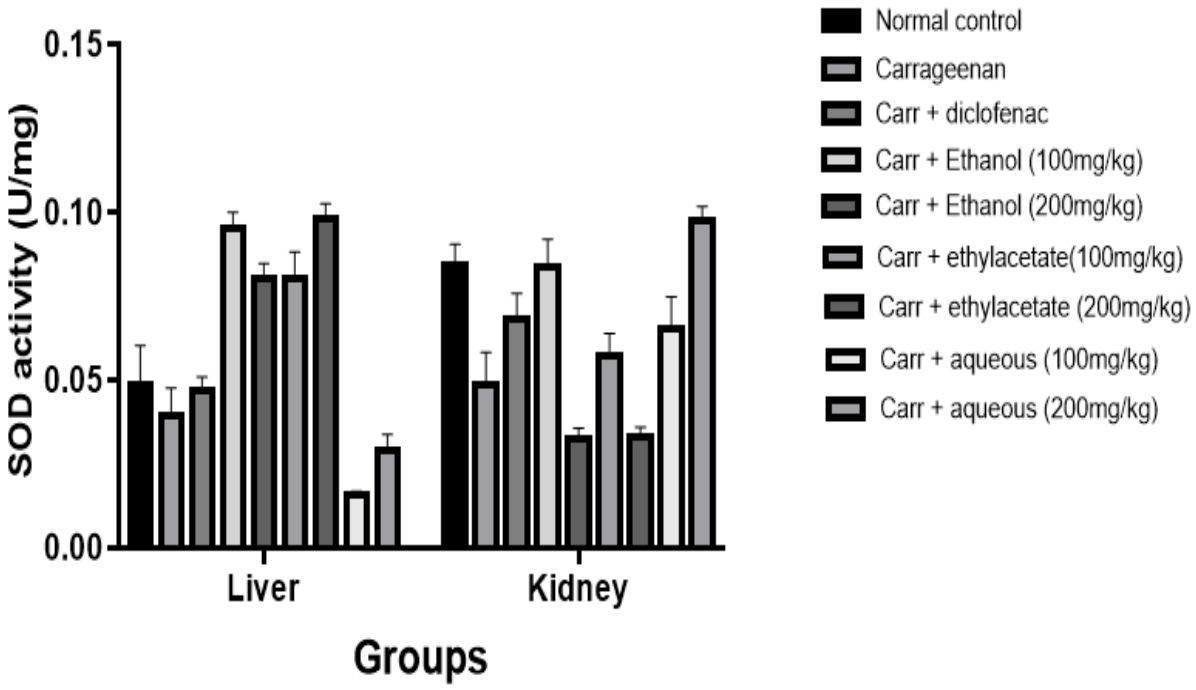

Figure 4.8: Superoxide dismutase activity of control and experimental mice with graded concentration of ethanol extract and fractions of *P. amarus*

Data are mean ± SD of replicate determination; n=4

Carr: Carrageenan

4.6 Effect of *P.amarus* ethanol extract and fractions on kidney and liver on the extent of Catalase activity in carrageenan induced inflammation in mice

From figure 4.9, there was no significant difference ($p > 0.05$) of liver catalase activity in mice administered with standard drug when compared to normal control, while the negative control recorded a higher catalase activity compared with the normal control.

However upon administration of all plant extract there were significant decreases ($p < 0.05$) in liver catalase activity when compared to negative control.

Also, from figure 4.9, there was a significant decrease ($p < 0.05$) of kidney SOD activity in mice administered with standard drug when compared to normal control, while the negative control recorded a lower SOD activity compared with the normal control. However upon administration of all plant extract there were significant increases ($p < 0.05$) in kidney SOD activity when compared to negative control.

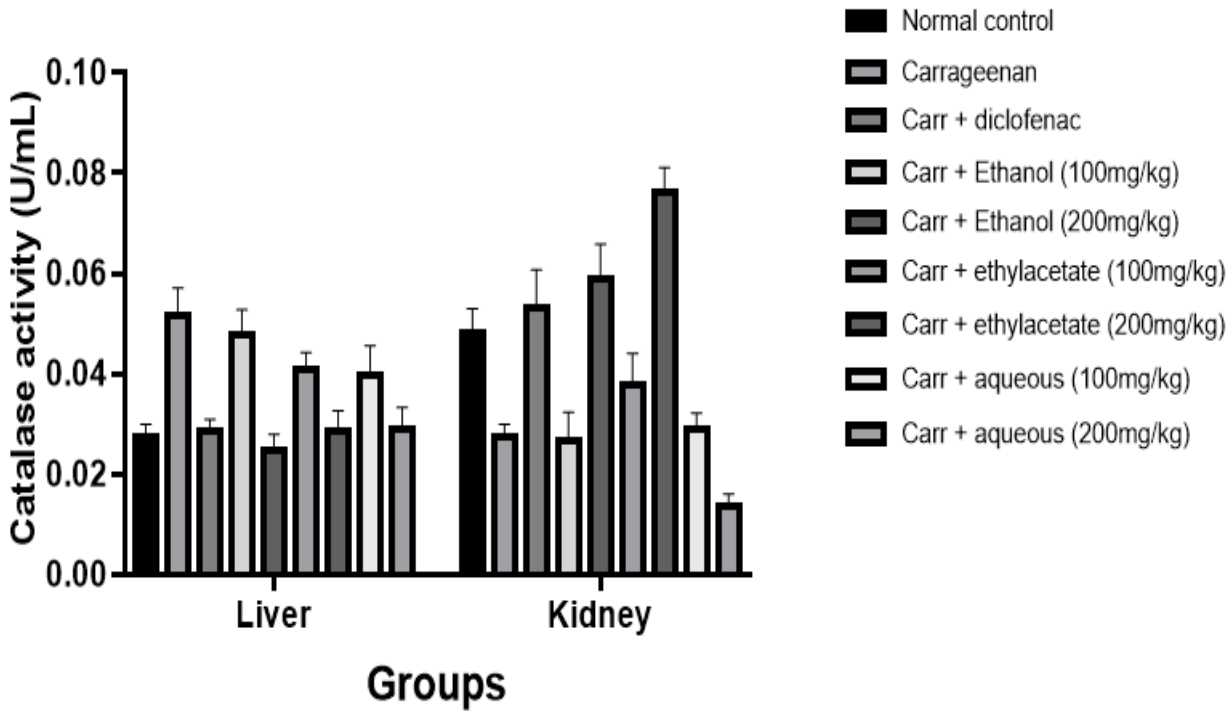

Figure 4.9: Catalase activity of control and experimental mice with graded concentration of ethanol extract and fractions of *P. amarus*

Data are mean ± SD of replicate determination; n=4

Carr: Carrageenan

4.7 Effect of *P.amarus* ethanol extract and fractions on liver and kidney reduced Glutathione concentration in carrageenan induced inflammatory mice

Figure 4.10 shows that there was no significant change in liver GSH concentration in mice given the standard medication compared to the normal control ($p > 0.05$), however the negative control had a greater GSH concentration than the normal control. When

compared to the negative control, however, there were significant decreases ($p<0.05$) in liver GSH concentrations after administration of all plant extracts.

In addition, as shown in figure 4.10, there was a significant drop ($p<0.05$) in kidney GSH concentration in mice given the standard medication compared to the normal control, but the negative control had a greater GSH concentration. However upon administration of all plant extract there were significant decreases ($p < 0.05$) in kidney GSH concentration when compared to negative control.

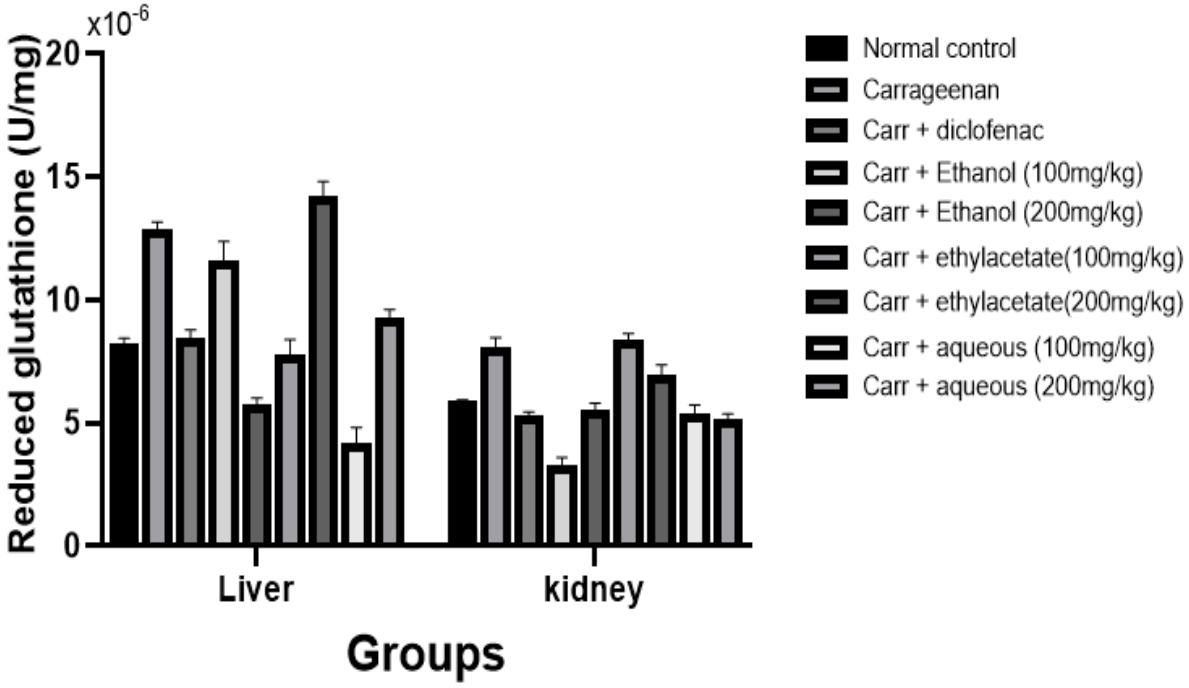

Figure 4.10: Reduced levels of glutathione concentration of control and experimental mice with graded concentration of ethanol extract and fractions of *P. amarus*

Data are mean ± SD of replicate determination; n=4

Carr: Carrageenan

4.8 Discussion

Phyllanthus amarus is a weed that is widely known for its medicinal properties. P. amarus has been used to cure a variety of ailments in the past, including jaundice, kidney stones, fever, fertility concerns, and menstrual cycle problems. Antimalarial, anticancer, antioxidant, and other pharmacological properties have been identified for the plant. The

effect of plant extracts from P. amarus on the lipid profile and antioxidant activity of carrageenan-induced inflamed mice was studied in this work.

The treatment of various fractions of the plant extracts to carrageenan-induced mice resulted in a significant ($p< 0.05$) reduction in paw sizes. This indicates that P. amarus has anti-inflammatory properties. Thus, the existence of secondary metabolites in which the plants may be able to limit the synthesis of prostaglandins by cyclooxygenase-2 suggests the presence of secondary metabolites in which the plants may be able to inhibit the production of prostaglandins by cyclooxygenase-2 (Arul et al. 2012).

This study found a substantial increase ($p>0.05$) in serum and liver triglycerides in the negative control group, but a drop in concentration when Ethanol extract (100 mg/kg and 200 mg/kg) and ethylacetate fraction (200 mg/kg) were given, implying that these extracts have lipid-lowering properties. The observed impact could be related to the plants' ability to reduce cholesterol production enzyme activity, therefore alleviating hyperlipidemia. This could also indicate that the *P. amarus* plant plays a preventive role against degenetrate sickness (Nwankpa et al. 2012).

The injection of ethanol extract (100 mg/kg and 200 mg/kg), ethylacetate (100 mg/kg and 200 mg/kg), and aqueous (100 mg/kg and 200 mg/kg) fractions of *P. amarus* resulted in a substantial ($p<0.05$) rise in the liver and blood HDL-cholesterol concentration when compared to normal control. These findings suggest that aqueous fractions could be employed as an anti-atherogenic medication to treat atherosclerosis (Oyewole et al. 2013).

The reduction in liver and kidney MDA concentrations in the groups treated with ethanol extract (100 and 200 mg/kg) and ethyl acetate extract (100 and 200 mg/kg) compared to the negative and normal control suggests that *P.amarus* shows that ethanol and ethyl acetate extract have the tendency to stimulate maintenance and survival through antioxidant defense mechanisms or signaling pathways that can help upregulate antioxidant proteins in adaptive stress responses (Yin et al . 2011).

The administration of ethanol extract (100 mg/kg and 200 mg/kg) and ethylacetate (100 mg/kg and 200 mg/kg) fractions of *P. amarus* resulted in a significant increase ($p <0.05$) in liver and kidney SOD activity when compared to the normal control; and administration of aqueous extract (100 mg/kg) resulted in an increase in kidney SOD activity. Thus, by converting superoxide radicals to hydrogen perioxide, the plant extract could operate as a defensive mechanism in the body, reducing oxidative stress and reducing oxidative stress (Antonio et al. 2006). Superoxide dismutase (SOD) converts superoxide anion radicals

produced in the body to hydrogenperioxide thereby reducing thereby reducing the superoxide anions interating with noitric oxide to form peroxynitrite.

When 100 mg/kg ethanol, ethylacetate (100 mg/kg and 200 mg/kg), and aqueous (100 mg/kg and 200 mg/kg) fractions of P. amarus were administered to mice, the liver and kidney catalase concentrations increased significantly ($p<0.05$), suggesting that catalase could be used as a therapeutic agent in oxidative stress-related neurological diseases (Jin et al .2001) Catalase is a key player in controlling cellular hydrogen peroxide levels and protecting cells from oxidative stress (Gaetani et al. 1996).

When 100 mg/kg ethanol, 200 mg/kg ethylacetate, and 200 mg/kg aqueous fraction of P. amarus were administered, the liver GSH concentration increased significantly (p 0.05) when compared to the normal control. This indicates that the extract does not induce renal damage because GSH levels in the liver have increased. GSH reductase is a family of enzymes that help to keep glutathione in its reduced state. They are Flavin adenine dinucleotide (FAD)-dependent. Because of the presence of Flavin adenine dinucleotide, the plant extract could be used to treat gluthathione reductase deficits (Deponte, 2013; Branwell and Grunden, 2015).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study showed and confirmed that *P. amarus* extract and fractions possess anti-inflammatory and hypolipidemic activities. Therefore, *P. amarus* fractions and extracts may be a source of novel hypolipidemic and anti-inflammatory agents in pharmaceutical drug development. Findings in this study indicate that *P. amarus* may have good antioxidant properties which could be helpful in prevention of some diseases.

5.2 Recommendations

Further study is required to elucidate the mechanism of hypolipidemic activity of *P. amarus* and isolation of anti-oxidant activity or agents from *P. amarus* in production of novel drugs in pharmaceutical industry.

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APPENDIX

Appendix I: Result for Cholesterol assay on liver sample

Group 1		Group 2		Group 3		Group 4	
Absorbance	Conc(mg/dl)	Absorbance	Conc(mg/dl)	Absorbance	Conc(mg/dl)	Absorbance	Conc(mg/dl)
0.027	31.1	0.445	513.3	0.150	173.0	0.048	55.4
0.024	27.7	0.498	574.4	0.170	196.1	0.063	72.6
0.022	25.4	0.501	577.9	0.165	190.3	0.059	68.1
	28.1		555.2		186.5		65.4

Group 5		Group 6		Group 7		Group 8		Group 9	
Absorbance	Conc(mg/dl)	Absorbance	Conc(mg/dl)	Absorbance	Conc(mg/dl)	Absorbance	Conc(mg/dl)	Absorbance	Conc(mg/dl)
0.513	591.7	0.027	31.1	0.401	462.5	0.431	497.1	0.604	696.6
0.519	598.6	0.028	32.3	0.390	449.8	0.420	484.4	0.693	799.3
0.501	577.9	0.038	43.8	0.355	409.5	0.392	452.1	0.681	785.4
	589.4		35.7		440.6		477.9		760.4