EVALUATION OF ANTI-INFLAMMATORY EFFECTS OF AQUEOUS LEAF EXTRACT OF *SYZYGIUM GUINEENSE* ON CARRAGEENAN-INDUCED PAW OEDEMA IN ADULT MALE WISTAR RATS

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DECLARATION

I declare that the work in the Dissertation entitled "Evaluation of Anti-inflammatory Effects of Aqueous Leaf Extract of *Syzygiumguineense* on Carrageenan-induced Paw Oedema in Adult Males Wistar Rats" has been carried out by me in the Department of Human Physiology, under the supervision of Dr. M. B. Akor-Dewu and Dr. I.G. Bako.All information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

_____ _____

ABBAS Ibrahim Date

CERTIFICATION

This dissertation entitled "Evaluation of Anti-inflammatory Effects of Aqueous Leaf Extract of *Syzygiumguineense* on Carrageenan-induced Paw Oedema in Adult Males Wistar Rats" by Abbas Ibrahim, meets the regulations governing the award of the Degree of Master of Science in Human Physiology, Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the memory of my late parents, Mal. Abbas Ibrahim Hamzah, and Khadija Abbas Ibrahim, whom until their deaths ensured their children getquality education.

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ABSTRACT

Inflammation is implicated in many disease conditions, such as cardiovascular diseases, diabetes mellitus and cancer. *Syzygiumguineense*has been reported to contain active phytochemicals that may inhibit the inflammatory responses, decrease the levels of inflammatory mediators and attenuate the abnormal formation of reactive oxygen metabolites. The aim of this study is to determine the Anti-Inflammatory Effects of Aqueous Leaf Extract of *Syzygiumguineense*on Carrageenan-induced Paw Oedema inAdult Male Wistar Rats.At the beginning of the study acute toxicity studies was carried out, which showed that the aqueous leaf extract of *Syzygium guineense* is safe to use since no mortality was recorded and no signs of toxicity was developed by the animals in each phases of acute toxcity studies. The preliminary phytochemical analysis was also conducted and variuos active agents were present in aqueous leaf extract of *Syzygium.*Thirty albino wistar rats were used for the study, the animals were divided into six (6) groups of five (5) rats each. The animals were grouped as follows: Group I received normal saline 1mg/kg only. Group II receive carrageenan injection only. Group III wasadministered with Diclofenac 10mg/kg orally. Group IV receieved 250mg/kg, Group V received 500mg/kg, and Group VI received 1000mg/kg of*Syzygiumguineense* per orally. One hour after the administrations, each rat except group I, was injected with 0.1ml of carragenan (1%w/v) into the subplanter region of the right hind paw. All the three doses of aqueous leaf extract of *Syzygiumguineense*, and Diclofenac 10mg/kgsignificantly ($P \leq 0.05$) inhibited the Carrageenan induced paw oedema after 3 hours. The anti-oxidant enzymes, SOD,CAT and GSH levels were not significantly ($P \leq 0.05$) increased by*Syzygiumguineense*compared to carrageenan group, but the 250mg/kg dose increase the SOD and CAT levels compared to normal saline group. The 250mg/kg of*Syzygium guineense* caused statistically significant ($P \leq 0.05$) increase in Neutrophil countscompared Carrageenan and normal

saline groups. The Eosinophil counts were statistically significant (P≤0.05) increased by 1000mg/kg of *Syzygium guineense* compared to Carrageenan and normal saline groups. While the monoctytes counts were statistically significant (P≤0.05) decreased compared to control groups. The results of this study shows that *Syzygiumguineense*has anti-inflammatory effects due to decrease in paw oedema andlittle antioxidant effects.

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ACE - Angiotensinogen Converting Enzyme

ANOVA- Analysis of Variance

CAM-1 - Intercellular Adhesion Molecule-1

CAT - Catalase

C3a - Complement 3a

C3b - Complement 3b

C5a - Complement 5a

COX - Cyclooxygenase

CRP - C reactive protein

CVD - Cardiovascular Disease

DM - Diabetes Mellitus

DMARDs - Disease-modifying antirheumatoid drugs

DNA - Deoxyribonucleic acid

eNOS - Endothelial Nitric Oxide Synthase

GSH - Reduced glutathione

H_2O_2 - Hydrogen Peroxide

HNO_2 - Nitrous Oxide

HOCl - Hydrochlorous Acid

HRO_2^- - Hydroperoxyl

ICAM-I - Intracellular Adhesion Molecule-1

IgG - Immunoglobulin G

IL-1 - Interleukin 1

IL-6 - Interleukin 6

IL-8 - Interleukin 8

IL-10 - Interleukin 10

iNOS - Inducible Nitric Oxide Synthase

JNK - c-Jun N-terminal Kinase

JAK/STAT - Janus kinase/signal transducers

Jak2 - Janus kinase 2

LT-B4 - Leukotriene B4

MAC - Membrane attack complex

MDA - Malondialdehyde

MAPK - Mitogen- activated protein kinase

MPO - Myeloperoxidase

NADPH- Nicotinic Acid Adenine Dinucleotide Phosphate

nNOS - Neuronal NOS

NO_2^- - Nitrogen Dioxide

NO - Nitric Oxide

nNOS - Neural Nitric Oxide Synthase

O_2^- - Superoxide

OH - Hydroxyl

$ONOO^-$ - Peroxynitrite

OS - Oxidative Stress

NSAIDs- Non steroidal ant-inflammatory drugs

RBC - Red blood cells count

RONOO- Alkyl Peroxynitrates

ROS - Reactive Oxygen Species

RNS-Reactive Nitrogen Species

RO_2 - Peroxyl

PPAR- Peroxisome proliferator-activated receptor

PECAM-1- Platelete-Endothelial Cell Adhesion Molecule-1

PMNS - Polymorphonuclear Neutrophils

SOD - Superoxide Dismutase

TBARS- Thiobarbituric Acid Reactive Substances

TNFα - Tumour Necrosis Factor Alpha

VEGF - Vascular Endothelial Growth Factor

VCAM-1- Vascular cell adhesion molecule-1

WBC - White Blood Cells Counts

CHAPTER ONE

1.0INTRODUCTION

For over a thousand decades plants extracts have been used for treatment against of ailmentssuch as malaria, typhoid, jaundice, hyperthermia, skin irritations, dysentery, anaemia, and gonorrhea(Idu *etal.,* 2010). About 80% of African people depend on herbal preparations for treatment of various ailments, especially when modern medicine is inaccessible (Elisha *etal.,* 2016). There is growing interest in different plant species nowadays due to the fact that many modern drugs are derived from plant sources, many people believe that natural products are safer and more effective than using pharmaceutical drugs even though not all herbal remedies are without toxic metabolites. The major problem affecting traditional remedies are lack of standardization and absence of safety regulations (Raphael, 2011).

Different parts of plants are used for treatment of particular ailments, and some factors must be considered such as botanical nature, chemical constituents, storage, and geographic factors (Antwi-baffour *etal.,* 2014). The major phytochemicals in plants that play a very significant role on inflammation are polyphenols from berries, fruit and vegetables (Ez Zoubi *etal.,* 2015).

Syzygium is the genus of the family myrtaceae which consist of about 500different speciessuch as the *syzygium guineense* that is very common in African countries.Thegenus is represented in Nigeria by three species;*Syzygium rowlandii,Syzygium owariense,* and *Syzygium guineense*.*Syzygium guineense* bear several names in different languages; Malmo in Hausa, Asurahi in Fulani, Mho in Tiv, and Adere/Ori in Yoruba (Keay *et al.,* 1964).

Syzygium guineense leaves have immunomodulatory, anti-oxidant, and anti-inflammatory properties (Tadesse and Wubneh, 2017). The plant has a lot of medicinal values such as treatment of asthma, tuberculosis, chronic diarrhea, cough, dysentery, malaria, amenorrhea, wounds, ulcers, rheumatism and infections(Abok and Manulu, 2016). The antioxidant properties of *Syzygium guineense* are due to its phenolic compounds revealed by phytochemical studies, which shows that the extracts of *Syzigium guineense* possess antioxidant propertiesand could serve as free radical inhibitors or scavengers, acting possibly as primaryantioxidants (Pieme *et al.*, 2014).

Inflammation is a response to cellular injury induced by physical stress, infectious agents, toxins, and other factors. Inflammation can be beneficial in case of acute, immune response to harmful conditions such as traumatic tissue injury or invading agent. This response also facilitates the repair, turnover, and adaptation of many tissues (Claudio and Judith, 2014). Chronic inflammation is slow and persistent and can cause tissue destruction and neurodegenerative diseases. Inflammatory response is part of the innate immune response. After an injury, the acute response involves macrophage activation, which leads to production of mediators such as histamine, macrophages and other inflammatory cellsthat generate a great amount of growth factors, cytokines, reactive oxygen and nitrogen species that may cause DNA damage. If the macrophages are activated persistently, theymay lead to continuous tissue damage.). Swelling of the tissues because of excess interstitial fluid is known as oedema. Thus the familiar swelling or oedema that accompanies inflammation is due to histamine induced vascular changes (Sherwood, 2006).

Anti-inflammatory drugs act by inhibiting the Cyclooxygenase (COX) enzymes that prevent the formation of inflammatory mediators such prostaglandins and thromboxane (Rivzi*et al.*, 2017).

Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and anti-histamines are commonly used to treat some inflammatory problems, and pain. Some NSAIDs act by inhibiting the cyclooxygenase enzyme to interfere with the production of prostaglandins. The other possible mechanism for action of NSAIDs are inhibition of chemotaxis, down-regulation of interleukin production, decreased production of free radicals, and interference with calcium-mediated intracellular events (Katzung*etal*.,2010

Oxidative stress is a phenomenon associated with the action of free radicals and reactive metabolites in organisms. Free radicals participate in a large number of subsequent reactions in which other reactive metabolites are formed. They are derived from basic radical molecules, such as superoxide anion radical,or nitric oxide (Durackova, 2009). An imbalance between oxidants and antioxidants cause oxidative damage to biological molecules. This damage if not controlled can lead to the development of a number of illnesses. Moreover, when overproduced, reactive oxygen species attack tissue and cause inflammatory response by production of pro-inflammatory mediators and chemotactic factors, which lead to chronic inflammation (Hamama *et al.,* 2016). Depending on the type of oxidants, intensity and time of redox imbalance as well as on the type of cells, oxidative stress can play a role in the regulation of other important processes through modulation of signal pathways, influencing synthesis of antioxidant enzymes, repair processes, inflammation, apoptosis and cell proliferation (Durackova, 2009). Carrageenan is a polysaccharide found in edible red seaweeds. Carrageenan-induced paw oedema is the most widely used method for anti-inflammatory study. It involves administering an immunomodulatory substances, such as carrageenan, ovalbumin, or formalin into a right hind paw of the animals. But in the case of mouse part of ear is also used (Necas and Bartosikova, 2013).

Figure 1.1: *Syzygium guineese* Fruits and Leaves (Hyde *et al.*, 2017)

1.1Statement of The Research Problem

Acute inflammation help the body to successfully deal with foreign agents in the body, but chronic inflammation can lead to the development of chronic illnesses such as cardiovascular diseases, diabetes, pulmonary diseases, and cancers (Mohan, 2013).Diseases associated with inflammation are among the global burden of death worldwide and their treatment with conventional drugs is very difficult (Lawrence and Gilroy,2006). Some of the diseases are irresponsive to the drugs, and sometimes treatments may lead to complications. Nearly most of the anti-inflammatory drugsparticularly the non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and anti-histamines are associated with serious side effects, commonlysedation, fatigue, loss of appetite, vomiting, and urinary retention (Lorato, 2016). Some of the drugs are unaffordable because of their high cost.

1.2Justification of The Study

Plants have been used traditionally for the management of pain and inflammation. Medicinal plants are available in many places and they are cheaper than anti-inflamatory drugs. Most of them are safe to use and they are not associated with major side effects or complications. Many studies have been carried out on *Syzygium guineense,* including its anti-microbial, anti-Malarial, and anti-diabetic effects, however, there is little information on its anti-inflamatory effects. *Syzygium guineense* contains many phytochemicals such as tannins, steroids, triterpenes, anthocyanin,

Leocoanthocyanins, saponins, and flavonoids (Tshibangu, 2011).Therefore the need to investigate the anti-inflammatory and antioxidant properties of aqueous leaf extract of *Syzygium guineense.*

1.3Aim and Objectives of the Study

1.3.1 Aim of the study

This study is aimed at investigating the Anti-inflammatory Effects of Aqueous Leaf extract of *Syzygium guineense* on Carrageenan-induced Paw Oedema in Adults Male Wistar Rats.

1.3.2 Objectives of the study

The objectives of this study are to;

(i) determine the protective effects of *Syzygium guineense* on Carrageenan induced paw oedema

(ii) assess some biomakers of inflammation such as Interleukin 6 (IL-6), and C reactive protein (CRP).

(iii) determine the differential white blood cell counts.

(iv) to assess oxidative stress biomarkers such as Superoxide dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH) and marker of lipid peroxidation; Malondialdehyde (MDA).

1.4Research Hypothesis

Aqueous leaf extract of *Syzygium guineense* does not have any Anti-inflammatory Effects on Carrageenan-induced Paw Oedema onAdult Male Wistar Rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Syzygium guineense*

Syzygium is a genus under Myrtaceae, which is a large family of trees or shrubs. They are floweringplants that consists of about 1100 species, found in many regions including Africa, Madagascar and south Asia. *Syzygium guineense* is one of the species under *Syzygium* genus. It has a very wide distribution in many regions of Africa (Tshibangu, 2011). In Nigeria it is represented by three species which differ from each other only by the nature of their habitat. The three species are *Syzygium rowlandii, Syzygium owariense,* and *Syzygium guineense,* there is another additional specie in this genus known as *Syzygium cumini.* Main feature that differentiate the different species is the presence of loosely branched terminal inflorecences and small greyish fruits. The plants is called by different names as follows; Hausa "*malmo",* Fulani "*asurahi"* Tiv " *mho"* andYoruba "*adere"* (Keay *etal.*, 1964).

Syzygium guineense are usually found in lowland rain forests, mountain rain forests, and swampy forests. It mostly grown in moist environments such as streams and Wadis. The tree require fresh, moist, and well-drain soils with high water table, but it sometimes grows in open woodlands. The height of *Syzygium guineense* is about 15-30 m high. The colour of its bark is variable depending on the age of the tree. Its leaves are narrow at both ends with length 5-17.5 cm, width 1.3-7.5 cm, they are simple and opposite. While the Flowers (filaments) has a diameter of 1.5 cm. Seeds are 1.3-1.4 cm in diameter, yellowish to brownish and rounded (Orwa *etal.*, 2009).

Syzygium guineense has many traditional uses as remedy for menstrual cycle, malnutrition, debility, naso-pharyngeal affections, pain-killers,pulmonary troubles, laxatives, heart, skeletal structure, stomach troubles, pregnancy,diarrhoea, dysentery, arthritis, rheumatism, venereal diseases and anaemia. The fruits ofthis plant are eaten as meal (sauces, condiments, spices, flavourings), while its barks andwoods have different uses, such as building materials for carpentry and related applications; farming, forestry, hunting, fishing apparatus; fuel and lighting, musical instruments, games, toys and as ornamental plant (Tshibangu, 2011).

The Phytochemical study of the leaves of *syzygium guineense* by Tshibangu (2011), reveals the presence of some secondary metabolites such as alkaloids, cardiac glycosides, tannins, steroids, triterpenes, anthocyanin, Leocoanthocyanins, saponins, flavonoids. While Parakashtha *et al.* (2010), showed that there are two immunologically active polysaccharides in the leaves of *Syzigium guineense*, sg50A1 and sg50A2, which stimulate proinflammatory and inflammatory cytokines from both cells and dendritic cells.Gildyall *etal*(2010), also identified two immunologically active polysaccharide fractions; one of them is arabinogalactan type II polysaccharide, and the other which is a mixture of oligosaccharides of pectic type. The two polysaccharides stimulate the secretions of both proinflammatory and anti-inflammatory cytokines both from B cells and dendritic cells.

The result of acute toxicity test by Ezenyi *etal.* (2016), showed that the extract did not lead to any sign of toxicity, and none of the animals die within two weeks after administration of extract up to 5000mg/kg. According to Lorato (2016), all the haematological parameters such as WBC, RBC, PLT, HGB, HCT, MCV, MCH, and MCHC, all were within the physiological ranges after oral administration of hydromethanol leaf extract of *Syzygium guineense.* Itdid not alsocause significant

changes in the histology of the liver and kidney.But oral administration of the extract causea dose-dependent decrease in blood glucose, serum creatinine, cholesterol, triglycerides and serum HDL, in diabetic rats. It also cause reduction in serum levels of liver enzymes, total and direct bilirubin and albumin (Ezenyi *et al.,* 2016).But long term use of higher doses of *syzygium guineense* might be associated with tissue and structural damage of liver and kideys. And the grain cannot be used if it is contaminated due to its toxicity (Antonia *et al.,* 2002). Because the seed of the plant contains a lot of micro and macronutrients. In a mineral analysis of the *Syzygium guineense,* the result showed the presence of calcium, phosphorus, iron, potassium and Sulphur.The fruitalso contains the electrolytes such as Ca, Mg, Fe, K and P. The presence of considerable quantities of potassium explain why the fruit cause reduction of blood pressure (Pieme *et al.,* 2014).

In a study conducted by Djoukeng *et al*. (2005), 10 triterpenes were isolated from *Syzygium guineense* which showed antibacterial activity in the treated rats.Another study was carried out by Maregesi *et al.*(2016), on the antihelmintic effect of the ethanolic extract of the seeds of *Syzygium guineense*, showed a dose dependent effect compared to standard drug albendazole tablets. The extract shows a dose dependents suppression of malaria parasite, increase in mean survival time of the study mice and suppress the pathological effects of the parasite on mice. This indicates that other than direct parasiticidal effects, the plant may possess other pharmacologic benefits to the host: acting as analgesics, antipyretics, immune stimulators (Tadesse, and Wubneh, 2017).

Pieme *etal.* (2014), showed a correlations between polyphenol content of ethanol/water extracts and antioxidant capacity, which is higher than the antioxidant capacity of vitamin C.The redox properties of phenolic compounds (phenols, flavonoids and flavonols) is responsible for their antioxidant ability which enable them to chelate transition metals and free radicals. Also the

number and position of hydroxyl group determine the antioxidant ability of the phenolic compound in the extract, to act as free radical scavengers (Pieme *et al*., 2014).

2.2Inflammation

Inflammation is the response to injury of a tissue and its microcirculation and is a means by which the body with the aid of inflammatory mediators as well as other mechanisms to localizes or restrict microorganisms, damaged cells, and foreignparticles, to help the body to return to normal structure and function (Rubin and Reisner, 2008). The steps of inflammation involvesrecognition of the injurious agent, recruitment of leukocytes, removal of the agent, regulation (control) of the response, and resolution (repair).The outcome of inflammation is mainly to remove injurious substance, which lead to decrease in the reaction and repair of the damaged tissue. If the reaction does not fall there will continuous reaction which cause chronic inflammation (Kumar *etal.,* 2005).

Classification of inflammation depend on defense capacity and duration of inflammatory response. Acute inflammatory response lasts briefly and it resolves and healed within two weeks. Acute inflammation is termed fulminant inflammation when acute inflammatory response become severe. Chronic inflammation takes place when acute inflammatory response fail to remove injurious agent and the stimulus persists for a long period the chronic inflammation take place (Mohan, 2013)

2.21 Acute Inflammation

Acute inflammatory response lasts for minutes to weeks. The events taking place in acute inflammation are divided into two; vascular and cellular events (Reed *et al.,* 1960). Vascular events

taking place during acute inflammation are due to the change in microvasculature (arterioles, capillaries and venules). Haemodynamic changes and changes in vascular permeability are the earliest response during acute inflammation.The haemodynamic changes involves change in vascular flow and caliber of small blood vessels in the inflamed tissue. This lead to the development of triple response; red line, flare, and wheal (Posadas *et al.,* 2004).The mechanism for increased vascular permeability include endothelial contraction orimmediate-transient response, mediated by histamine, bradykinin, and leukotrienes. Mediators such as tumour necrosis factor (TNF), and interleukins especially interleukin 1 (IL-1), lead to occurrence of endothelial cell retraction or delayed response (Mohan, 2013).

The cellular phase of inflammation is divided into: exudation of leucocytes and phagocytosis. Modification of the formed element of blood bring the leucocyte close to the wall of the blood vessels. Adhesion of leucocytes to the endothelial cell lining is aided by action of adhesion molecules such as Selectins, Integrins, and Immunoglobulin gene superfamily adhesion molecule . Selectin are divided into various subtypes including s- Lewis X molecule, P-selectin, E-selectin and L-selectin". Immunoglobulin gene superfamily adhesion molecule, include intercellular adhesion molecule-1 (CAM-1), Vascular cell adhesion molecule-1 (VCAM-1), Platelete-endothelial cell adhesion molecule-1 (PECAM-1) or CD31.The common chemotactic factors or chemokines that mediate movement of cells to the site of injury include: leukotriene B4 (LT-B4), Component of complement system (C5a and C3a), Cytokines (IL-8), and some soluble bacterial products (Mohan, 2013).

The phagocytic cells: polymorphonuclear neutrophils (PMNS), and circulating monocytes and fixed tissue phagocytes (macrophages), they recognize microorganisms due to the presence of surface receptors such as mannose receptors and scavenger receptors which identify the protein coats, known as opsonins at the surface of microorganisms. Various opsonins have been identified. the most notable ones are; *IgG opsonin, C3b opsonin* and *Lectins.* Microorganisms are killed by generation of reactive oxygen metabolites, especially H_2O_2, formed from reduction of oxygen by NADPH-oxidase. The granules of the leucocytes contain enzymes such as protease, trypsinase, phospholipase and alkaline phosphatase, which are released during inflammatory response to kill the microorganisms(Kumar *et al.,* 2005).The Leucocytes also destroy the microorganisms by non-oxidative processes because some enzymes such as lysosomal hydrolases, permeability increasing factors, cationic proteins (defensins), lipases, proteases, DNAases, they kill bacteria by lysis within the phagososmes. Extracellularly microorganisms are killed by degranulation of macrophages by the release of enzymes present in the granules of the macrophages. Antibody –mediated lysis and cell-mediated cytotoxicity are the immune-mediated microbial killings taking place outside the cell (Mohan,2013).

2.2.2 Chronic Inflammation

Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occur at the same time. When the acute inflammatory response failed to resolved the inflammation and the stimuli does not stop, the situation turn into chronic inflammation. Chronic inflammation is caused by persistence of infections by microbes, leading to T Lymphocytes-mediated immune response or delayed type hypersensitivity. It also results from immune-mediated inflammatory diseases (hypersensitivity diseases). When immune reactions is developed against body's own

tissues is termed autoimmune diseases, if it persists for a very long time it leads to chronic diseases such as rheumatoid arthritis and inflammatory bowel disease. Allergic diseases such as bronchial asthma, are caused by immune reactions against environmental agents (Kumar, 2005).

Chronic inflammation is subdivided into two types: Non-specific Chronic inflammation and Specific Chronic Inflammation.Non-specific chronic inflammation occur when the irritant or stimuli does not cause a specific inflammatory reaction, and it usually healed by fibrosis.In pecific Chronic Inflammation some inflammatory agents cause a particular histological tissue changes that are present in diseases such as tuberculosis, leprosy, and syphilis. Based of histological changes chronic inflammation is divided into.

i. Chronic non-specific inflammation; In this type of inflammation there is infiltration of non-specific inflammatory cells. It is also called chronic suppurative inflammation which is characterized by infiltration of polymorphs and abscess.
ii. Chronic granulomatous inflammation;Granulomas are formed in this type of inflammation. It takes place in diseases such as tuberculosis, leprosy, syphilis, actinomycosis, sarcoidosis (Mohan, 2013).

2.2.3 Mediators of Inflammation

In order for a particular mediator bring about its function it bind with specific receptors on target cells. Their actions on target cells is direct enzymatic or through toxic activities. Once the target cells are stimulated, they release secondary effector molecules, to cause either increase or decrease in response. The activities of mediators are tightly regulated, because they are quickly destroyed or removed when ever their actions is over.Mediators of inflammation are divided into cell-derived

mediators and plasma derived mediators. The mediators remain inactive in the granules, they become active when they reach the site of inflammation (Kumar *etal.,* 2005).

2.2.3.1 Plasma Derived Mediators

The activities of plasma derived mediators depend on the actions of proteases enzymes on three cascades such as the coagulation cascade and fibrinolytic system, kinin generation, and the complement system.

Coagulation and Fibrinolytic system

Negatively charged surfaces such as basement membranes, proteolytic enzymes, bacterial lipopolysaccharides, and foreign materials activate the Hageman factor (clotting factor XII). After its activation it leads to activation of; (i) intrinsic coagulation mechanism (ii) fibrinolysis and production of plasmin and plasmin-derived peptides (iii) kallikrein as well as kinins and (iv) activation of the alternate complement pathway kinins. The major roles of kinins is stimulating the release of many inflammatory mediators such as prostanoids, cytokines (TNF-α, Interleukins), and nitric oxide (NO). Since kinins are short-lived they are degraded by kininases and become inactive(Rubin and Reisner, 2008).

Complement system

Formation of membrane attack complex (MAC) is the main target of complement activation. Nearly most of the products of complement activation known to play an inflammatory role include:Anaphylatoxins (C3a, C4a, C5a), Opsonins(C3b, iC3b), Proinflammatory molecules(MAC, C5a). These activate leukocytes and tissue cells to generate oxidants and cytokines and induce

degranulation of mast cells and basophils. The complement system is activated by three convergent pathways termed classical, mannose-binding lectin (MBL), and alternative pathways.

Classical pathway is activated by antigen-antibody complex, products of bacteria and viruses, proteases, urate crystals, apoptotic cells and polynucleotides (Kumar *et al.*, 2005). The protein components of classical pathway ranges from C1 to C9. Some microbes have terminal containing mannose groups which bind with mannose-binding lectin a member of the family of calcium-dependent lectins. It has a wide range of functions which resemble the roles of immunoglobulins, IgM, IgG, and C1q. The activation of alternative pathway is caused by products of microorganisms and foreign materials. The activation take place at the level of C3 to form C3b (Rubin and Reisner, 2008).

2.2.3.2 Cell-Derived Mediators

Vasoactive amines

The vasoactive amines that play role in the early inflammatory response are histamine, 5-hydroxytryptamine (5-HT) or serotonin and neuropeptides. Histamine are produced by the granules of mast cells, basophils, and platelets. Its release is stimulated by various factors; temperature, chemicals, irradiation, anaphylatoxins, and interleukins. The main roles of histamine include; vasodilatation, increased vascular permeability, itching and pain. 5-hydroxytryptamine (5-HT) or serotonin are produced by many structures such as chromaffin cells of GIT, spleen, nervous tissues, mast cells and platelets. Its actions is closely related to those of histamine but it is not as active as histamine.Neuropeptides are small peptides generated by the tissues of central and peripheral

nervous systems. Such peptides include substance P, neurokinin A, vasoactive intestinal polypeptide (VIP) and somatostatin. They have some proinflammatory actions such as; increased vascular permeability, transmission of pain stimuli, and mast cells degranulation (Mohan, 2013).

Arachidonic acid metabolites

The arachidonic acid metabolites they mediate nearly every step of inflammation, they are produced by leucocytes, mast cells, endothelial cells and platelets. Its action is short lived as it will be degraded immediately after its action is over. The products of cyclooxygenase pathway include prostaglandins; PGE_2, PGD2, $PGF_2\alpha$, PGI_2, and thromboxane A_2.

Lipoxygenase pathway proceeds by the action of enzyme, 5-Lipoxygenase toform Leukotrienes. Some of the subtypes of Leukotrienes areleukotrienes (LTA_4), LTB_4 LTC4, are derived fromLTD_4 and LTE_4. Their major actions include; vasoconstriction, bronchospasm, and increased vascular permeability.Lipoxins are derived from activated platelets which are adherent to the leucocytes area. The main functions of Lipoxin is protecting the tissues against inflammation by inhibition of neutrophil chemotaxis, and adhesion to the endothelium (Kumar *et al.,* 2005).

Platelet activating factor

Platelet activating factor is an acetyl glycerol ether phosphocoline derived from membrane phospholipids of neutrophils, monocytes, basophils, endothelial cells, and platelets by the action of phospholipase A_2.It has a broad spectrum anti- inflammatory effects by acting through a G-protein coupled receptors. PAF promote inflammatoryreactions such as leucocyte adhesion, chemotaxis, leucocyte degranulation, and oxidative burs (Kumar *et al.,* 2005)

Cytokines

Cytokines are involved in the earliest immune and inflammatory reactions and later adaptive or specific immune responses to microbes. Interleukins have the ability to aid communications between many leucocytes, they act on leucocytes and many cells other than leucocytes. Chemokines are cytokines that are chemoattractants to leucocytes. Some important cytokines include;TNF-α, IL-1, IL-6interferon-γ (IFN-γ) and IL-12. The main effects of cytokines in inflammation is by endothelial activation through expression of adhesion molecules on endothelial cells to promote recruitment of leucocytes and generation of more of cytokines.Chemokines are proteins with very small molecular weight. They serve as chemoattractants for different leucocytes, leucocyte recruitment and activation in inflammation. Their action is through the G-protein coupled receptors. Chemokines include monocyte chemoattractant protein

1 (MCP-1) and macrophage inflammatory protein 1α (MIP-1α), RANTES (regulated on activation normal T expressed and secreted) which is chemotactic for memory CD4+ T cells and monocytes, and eotaxin which is chemotactic for eosinophils(Kumar *et al.,* 2005).

C-reactive protein (CRP)

C-reactive protein (CRP) is an acute inflammatory protein that is known to increase several times at the site of inflammation. It is synthesized mainly by liver and other structures such as smooth muscle cells, macrophage endothelial cells, lymphocytes, and adipocytes. Initially CRP was used as a marker of infection and cardiovascular diseases, but recently it appeared to play a significant roles in the inflammatory processes (Sproston, 2018). Release of CRP occurs in response to increased levels of inflammatory cytokines, especially interleukin-6 (IL-6). The normal average levels of CRP in serum in a healthy human is around 0.8 mg/L. Its highest concentrations are found in serum. CRP

plasma levels increase from around 1 μg/mL to over 500 μg/mL within 24–72 h of severe tissue damage such as trauma and progressive cancer. CRP does not only serve as marker of inflammation but it also plays a key role by an unclear mechanism.

2.2.4 Inflammatory Response Mechanisms

Chen *et al.* (2018), showed that inflammatory response involved activation of signaling pathways. It is these pathways that regulate levels of inflammatory mediators. Inflammatory response depend on nature of initial stimulus, and its location. All the responses pass through the following steps; recognition of stimuli by cell surface pattern receptors, activation of inflammatory pathways, release of inflammatory markers; and recruitments of inflammatory cells.

Pattern recognition receptor activation

The inflammatory response is initiated by pathogen-associated molecular patterns (PAMPs) from microorganisms. Activation takes place through germline-encoded pattern-recognition receptors (PRRs). One of PRR families are the Toll-like receptors (TLRs), The inflammatory response is mediated through Toll-like receptors (TLRs), which leads to nuclear translocation of transcription factors, the commonest amongst them is and NF-κB (Chen *et al.,* 2018).

Activation of inflammatory pathways

Receptor activation triggers important intracellular signaling pathways, including the mitogen-activated protein kinase (MAPK), nuclear factor kappa-B (NF-κB), and Janus kinase (JAK)-signal

transducer and activator of transcription (STAT) pathways. Activation of NF-κB transcription factor is critical for a wide range of processes such as immunity, inflammation, cell development, growth and survival. It is activated by a variety of stimuli including cytokines, ionizing radiation and oxidative stress.NF-κB acts at the crossroads of many signalling pathways. Inappropriate or excessive activation of NF-κB can lead to inflammatory diseases and cancers (Moynagh, 2015).

MAP kinases belongs to a large family of serine/threonine kinases, that form a highly integrated network required toachieve specialised cell functions controlling cell differentiation,cell proliferation, and cell death. The major class of MAP kinases is the JNK signalling pathway which is implicatedin a large variety of pathological conditions, including cancer,stroke, ischaemic heart disease, and inflammatory disorders. The other one is p38 MAP kinase pathway which is associated with inflammation,cell growth, cell differentiation, and cell death(Chen *et al.,* 2018). JAK-STAT pathway involves diverse cytokines, growth factors, interferons, and related molecules, such as leptin and growth hormone, and is a signaling mechanism through which extracellular factors can control gene expression. JAK/STAT signaling allows for the direct translation of an extracellular signal into a transcriptional response. Dysregulation of NF-κB, MAPK, or JAK-STAT activity is associated with inflammatory, autoimmune, and metabolic diseases, and cancer (Chen *etal.,* 2018).

2.2.5Diseases Associated With Inflammation

Cancer

About 25% of cancers are caused by inflammation and infections. Chronic inflammation causes various types of damage to nucleic acids, proteins and lipids through generation of ROS/RNS

generation, which causes tissue damage. The tissue injury then activate progenitor or stem cells which lead to regeneration. The mutations of stem cells due to generations ROS/RNS leads to the formation of cancer cells (Murata, 2018) .

Cardiovascular Disease

The great evidence for the relationship between cardiovcascular diseases and inflammation was due to the increase in circulating CRP inpatients with Myocardiac infarction. Other inflammatory mediators found to be increased in patients with cardiovascular disorders were IL-1, IL-6, and TNF-α. The inflammation causes endothelial dysfunction, oxidative stress in the vascular endothelial cells, and development of artherogenesis (Katsiari *et al.,* 2018).

Obesity and Diabetes mellitus

Increased levels of markers and mediators of inflammation and acute-phase reactants such as fibrinogen, C-reactive protein (CRP), IL-6, plasminogen activator inhibitor-1 (PAI-1), sialic acid, and white cell count is associated with incident of Type II diabetes mellitus. Also experiments showing that adipose tissue–derivedproinflammatory cytokines such as TNF-α could actually cause insulin resistance in experimental models. Also accumulation of lipids activate NADPH oxidase, which increases ROS production. This mechanism was shown to increase the production of TNF-α, IL-6, and MCP-1, and decrease the production of adiponectin (Shoelson *et al.,*2006).

Rheumatoid Arthritis

Rheumatoid arthritis is a chronic disease of unknown cause. The most common feature of RA is inflammatory arthritis of the peripheral joints. Other manifestations include haematologic, pulmonary, neurological and cardiovascular abnormalities. Antigenic exposure (e.g. infectious agent) leads to the release of cytokines, such as tumour necrosis factor (TNF-α), interleukins (IL-1 and IL-6). These cytokines activate endothelial cells, B lymphocytes and macrophages (Schiotis *et al.,* 2017). Activation of B-cells releases IgM antibody against IgG. IgG and IgM immune complexes trigger inflammatorydamage to the synovium, small blood vessels and collagen. Activated endothelial cells express adhesion molecules which stimulate collection of inflammatory cells. Activation of macrophages releases more cytokines which cause damage to joint tissues and vascularisation of cartilage. The damage and destruction of bone and cartilage leads to joint deformities (Mohan, 2013).

Inflammatory Bowel Disease (IBD)

Inflammatory bowel disease stands for two groups of diseases, the ulcerative colitis and Crohn's disease. It is caused by both immune and non-immune contributing factors. The chronic inflammation lead to release of inflammatory cytokines such as Cytokines linked with active IBD are IL-6, IL-1_ and TNF-which stimulate the production of CRP.

Chronic Obstructive Pulmonary Disease (COPD)

COPD is due to a prolonged inflammatory process in the airways, parenchyma and pulmonary vasculature. The above damages were caused by oxidative stress and activation of circulatory inflammatory cells. The most prominent features include an increased number of neutrophils, macrophages and T-lymphocytes , and amplified concentrations of proinflammatory cytokines such

as LTB4, TNF-α IL-1 and IL-8. Also the levels of CRP are higher in COPD patients when compared with healthy controls (Lavetti *et al.*,2013).

Asthma

Asthma is a chronic inflammatory disease which affects the lungs, and major parts of the respiratory tracts. This is due to the abnormal production of TH2 which lead to the elevation in the levels of interleukin-4 (IL-4), immunoglobulin E (IgE), and IL-5. the interstitial tissue is altered by fibroblast proliferation and differentiation, collagen deposition, and hypertrophy and hyperplasia of airway smooth muscle cells that also produce pro-inflammatory factors (Banno*et al.*,2018).

CNS Related Diseases

Inflammation of the CNS can cause cognitive impairment due to cytokines mediated interactions between neurons and glial cells which activates hypothalamicpituitary-adrenal (HPA) axis. Different inflammatory markers like TNF-α, IL-1, IL-6 and prostaglandins have been detected as targets in support of different CNS disorders such as stroke, traumatic brain injury, epilepsy, multiple sclerosis, Alzheimer's disease, schizophrenia, depression and Parkinsonism (Lavetti *et al.*,2013).

2.2.6Anti-Inflammatory Drugs

There are two primary goals treating inflammation: one of them is to reduce symptoms and maintain function. Secondly, inflammatory problems are also treated in order to slow or stop tissue damage. Nearly most of the nonsteroidal anti-inflammatory drugs (NSAIDs) bring about pain relief

for long time, but other groups of drugs were developed for treatment of different inflammatory conditions (Katzung *etal.,* 2012)

2.2.6.1Nonsteroidal Anti-Inflammatory Drugs (NSAIDS)

Nearly all NSAIDs are anti-inflammatory, analgesic, and antipyretic. Most of them are organic acids but sometimes they are chemically unrelated, and they share certain therapeutic actions and adverse reactions. The main therapeutic effects of NSAIDs is primarily because of their ability to inhibit COX enzymes and prostaglandin production. They do not inhibit lipoxygenase pathways of arachidonic acid metabolism. The major anti-inflammatory effects of NSAIDs is in the treatment of rheumatoid arthritis and osteoarthritis. While they cause symptomatic relief from pain and inflammatory diseases, but it cannot stop or modify progression of disease or tissue injury caused by inflammation (Brunton *etal.,* 2006).NSAIDs include salicylates and other compounds, some of them share many common characteristics. The choice of particular NSAIDs depend on the condition of the patient.

- ✓ Salicylates

Aspirin and sodium salicylates are the most commonly used salicylates. Aspirin inhibits COX-1 greatly than COX-2, while sodium salicylates is more selective for COX-1.

- ✓ Aryl and Heteroarylalkanoic Acid–Type Drugs

This group of drugs include indomethacin ibuprofen Sulindac, Tolmetin, Ketorolac and Diclofenac. Their selectivity for COX-1 and COX-2 varies from drug to drug (Craig and Stitzel, 2004).

- ✓ Sulfonylphenyl Derivatives

The drugs under this category include Celecoxib and rofecoxib. They are highly selective COX-2 inhibitors. They cause less side effects than other nonselective COX inhibitors.

- ✓ Oxicam-Type Drugs

Piroxicam is a common example in this group. It is a nonspecific COX inhibitor that has much higher affinity for COX-1 than COX-2. Plays important role in the treatment of rheumatoid arthritis and osteoarthritis.

- ✓ Fenamate-Type Drugs

Fenamate-type of drugs include Mefenamic acid and Meclofenamate sodium. Mefenamic acid is used for treatment of analgesia and primary dysmenorrhea, while Meclofenamate sodium is used for rheumatoid arthritis and osteoarthritis.

- ✓ Phenylbutazone-Type Drugs

The phenylbutazone-type drugs include phenylbutazone, oxyphenbutazone, antipyrine, dipyrone, and aminopyrine. The use of these drugs has decreased because of their propensity to cause blood dyscrasias (Craig and Stitzel, 2004).

2.2.6.1.1 Adverse Effects of NSAID Therapy

- ✓ Gastrointestinal effects of NSAIDs

Induction of ulcer by NSAIDs cause anorexia, nausea, dyspepsia, abdominal pain, and diarrhea. These symptoms may be related to the induction of gastric or intestinal ulcers. Inhibition of COX-1 enzyme cause the GIT to lose the protective effects of prostaglandins, PGI_2 and PGE_2. These two prostaglandins inhibit acid secretion, promote mucosal blood flow, and increase the secretion of mucus in the intestine.

- ✓ Cardiovascular effects

Inhibition of PGI_2 by selective COX-2, cause thrombosis, because the cardiovascular roles of thromboxane A_2 is depressed. NSAIDs cause loss of prostaglandin-induced inhibition of reabsorption of Cl^- and activities of ADH, which cause retention of salt and water. NSAIDs also cause increase in reabsorption of K^+ and decrease the availability of Na^+, and it end the suppression of secretion of renin by prostaglandins. Also inhibition of COX-2, lead to prostaglandins associated vasodilation and increase blood pressure (Brunton *et al.,* 2006).

- ✓ Analgesic Nephropathy

Long-term usage of NSAIDs lead to analgesic nephropathy, a condition characterized by slowly progressive renal failure, decreased concentrating capacity and sterile pyuria.

- ✓ Pregnancy and Lactation

NSAIDs cause delayed gestation, because some hours before parturition, the levels of prostaglandins $E_{2,}$ and $F_{2\alpha}$ increases in the myometrium.

- ✓ Hypersensitivity

Hypersensitivity reactions are associated with aspirin and NSAIDs include. Vasomotor rhinitis with watery secretions, angioderma, generalized urticarial, bronchial asthma, bronchoconstriction, flushing, hypotension and shock. Aspirin hypersensitivity reaction is due to the increase in biosynthesis of LTs as a result of diversion of AA to lipoxygenase metabolism (Brunton *etal.*, 2006).

2.2.6.2 Disease-Modifying Antirheumatic Agents

Disease-modifying antirheumatoid drugs (DMARDs) are drugs used for slowing the course of the disease, induce remission, and prevent further destruction of joints and other tissues. They are drug of choice intreatment of rheumatoid arthritis (Schiotis *et al.,* 2017). These include *methotrexate, hydroxychloroquine*. When the treatment with the traditional agent is not effective, newer groups are used. They are *leflunomide, anakinra,* andTNF-inhibitors *(adalimumab, etanercept, dalimumab, etanercept,* and *infliximab* (Finkel *etal.,* 2009).

2.2.6.3Glucocorticoids

Corticosteroids are drugs used for treatment of rheumatoid arthritis, vasculitis, systemic lupus erythematosus, Wegener's granulomatosis, psoriatic arthritis, giant cell arteritis, sarcoidosis, and gout (Katzung *etal.,* 2012).They bring about effects on many organ system, and they play many roles in inhibiting the cells of inflammatory mediators. They also decrease the expression of inflammatory cytokines such as COX-2 (Brunton *etal*., 2006).

2.2.6.4Inhibitors of Nitric Oxide Synthase

Overproduction of NO causes diseases such as arthritis, asthma, cerebral ischemia, Parkinson's disease, neurodegeneration, and seizures. Nitric oxide synthase (NOS) is the enzyme responsible for NO biosynthesis, and there are three main kinds of NOS isoforms; endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS). Depending on the clinical condition, decreasing NO levels is necessary, and excellent benefits might be achieved using NOS inhibitors. Commonest inhibitor of NOS is tilarginine, used in the treatment of myocardiac infarction (Ferreira and Serafim, 2017).

2.2.6.5 Peroxisome Proliferator Activated Receptors (PPAR) Agonists

PPAR exerts its anti-inflammatory activities in both the peripheral as well as the CNS glial and neuronal cells, where it regulates the expression of pro-inflammatory molecules such as iNOs, COX-2. According to Banno *et al.,* (2018)peroxisome proliferator-activated receptor (PPAR) are involved in asthma pathogenesis. PPAR agonists exert their anti-inflammatory effects primarily by suppressing pro-inflammatory mediators and antagonizing the pro-inflammatory functions of various cell types relevant to asthma. Experimental findings strongly support the potential clinical benefits of PPAR agonists in the treatment of asthma.

2.2.6.6 Angiotensinogen Converting Enzyme (ACE) Inhibitors

Independent of their blood pressure lowering effects, ACE inhibitors are thought to reduce vascular inflammation. ACE inhibitors are well-recognized to be helpful in reducing blood pressure and also in the regression of leftventricular hypertrophy, both in animal models of arterial hypertension as well as in humans with essential hypertension. In addition, it has also been shown that the treatment with ACE inhibitors could reverse the increased production of superoxide anions, NF-κB activation, expression of pro-inflammatory cytokines and reduced NO

synthesis in atherosclerosis. Captopril, an ACE inhibitor, has also been observed to function as an anti-inflammatory agent in hypertensive animals, and its anti-inflammatory activity has previously been shown in several other diseases such as colitis and arthritis (Lavetti *et al.,* 2013).

2.2.7.0 Anti Inflammatory Medicinal Plants

According to Antwi-Baffour *et al.,* (2014) the therapeutic potential of plants was investigated but there are needs for further study to improve their effects on various diseases. The chemical composition of medicinal plants varies depending on several factors, such as botanical species used,

chemotypes, the anatomical part of the plant used as well as the storage, sun, humidity, type of ground, time of harvest and geographic area.

Coriandrum sativum L.

Coriander is a plant used in the disorders of digestive, respiratory and urinary systems, as it has diaphoretic, diuretic, carminative and stimulant activity. Anti-inflammatory activity in ethanolic extract of *Coriandrum sativum L.* using carrageenan induced paw oedema in albino rats was evaluated by Mohan (2013). The anti-inflammatory activity is highest at 400mg/kg of Coriandrum sativum ethanolic leaf extract.

Beta Caryophyllene

Beta-Caryophyllene is a potent phyto cannabinoid which is reported to have anti-inflammatory activity. It is a sesquiterpene present in very large amounts in natural products e.g. clove oil, cinnamon leaves, and copaiba balsam, all of which have been used as natural remedies. According toVijayalaxmi *et al.* (2014)Beta-Caryophyllene significantly decreased paw volume and the arthritis in a carrageenan induced oedema model of inflammation.

Areca catechu

Areca catechu, commonly known as betel nut, is a very famous for its medicinal use in multiple disorders. It is also popular as a remedy against inflammatory disorders. Preliminary experiments by Mehmood*et al.*(2014) using a single dose (100 mg/kg) of Areca catechu and its respective fractions demonstrated an anti-inflammatory effect on carrageenan-induced edema in mice and rats. Its aqueous fraction exhibited free radical scavenging activity.

Citrus aurantium

Citrus aurantium has several uses such as for their appetizing, diaphoretic, antiseptic, analgesic and anti-inflammatory effects. Essential oil of Bergamot from *Citrus aurantium* was investigated by *Karacaet al.* (2007) for anti-inflammatory activity using carrageenan-induced rat paw oedema test and it was found to have strong anti inflammatory effects.

Babylonia zeylanica

Babylonia zeylanica is marine invertebrates with potential sources and the bioactive compounds such asalkaloid, ester, nitrogen, terpene and steroid. Its analgesic, antipyretic and anti-inflammatory effect study by Santhi *et al.*(2012)in Albino rats showed that all the doses showed good analgesic, antipyretic and anti- inflammatory effects.

Azadirachta indica

Azadirachta indica (Neem) is an evergreen tree, cultivated in various parts of the world. Neem, the versatile medicinal plant is the unique source of various types of compounds having diverse chemical structure. A study by Kanagasanthosh *et al.(*2015), indicates that oral administration of both the doses of Azadirachta indica leaves shows dose dependant improvement in the anti-inflammatory activity.

Aristotelia chilensis

Aristotelia chilensis serves as an ethnomedicinefor many years, and is used particularly as an anti-inflammatoryagent, kidneys pains, stomach ulcers; and diverse digestive ailments. Céspedes *et al.* (2010) demonstrated that the fruit and its constituents of have anti-inflammatory and gastroprotective and thus have great potential as nutraceuticals.

Anchomanes difformis

Almost all the plant parts *Anchomanes difformis* have been employed as purgatives;similarly, it is used for the treatment of dysentery, kidney pains, oedemas, urethral discharge and jaundice. The leaf extract of *Anchomanes difformis* was investigated by Adebayo (2014) for anti-nociceptive and anti-inflammatory effects in albino wistar rats and found that it exhibited some degrees of inhibition against albumin induced inflammation at a later phase.

2.2.7.1 Mechanism of Actions of Anti-Inflammatory Medicinal Plants

By Inhibition of Nitric Oxide Synthase

Natural compounds commonly found in foods may contribute to protect cellsagainst the deleterious effects of inflammation. These anti-inflammatory properties havebeen linked to the modulation of transcription factors that control expression ofinflammation-related genes, including the inducible nitric oxide synthase (iNOS), ratherthan a direct inhibitory action on these proteins. Natural compounds such as silibinin and cyanidin-3-rutinoside and other flavonoids showed the inhibitory activities of iNOS (Maldonado-Rojas and Olivero-Verbel, 2012).

Lipoxygenase Inhibiting Activity

LOXs are associated with several inflammation-related diseases such as arthritis, asthma, cardiovascular, kidney, skin and allergic diseases, neurodegenerative disorders, cancer and metabolic syndrome. Chung *et al.,* (2009) showed LOXs inhibitory activities by the following plants parts; barks of *Agelaea borneensis*, *Chisocheton polyandrus*, *Garcinia cuspidate*, and leavesof*Timonius flavescens*.

Inhibition of Cyclooxygenase Activity

Alberto *et al.,* 2009 determined the anti-inflammatory activity of extracts of four medicinal plant species (*Baccharis incarum*, *B. boliviensis*, *Chuquiraga atacamensis*, *Parastrephia lucida*). The extracts inhibit cyclooxygenase enzymes (COX-1 and COX-2) and also inhibited the production of PGE2.

By Inhibition of Inflammatory Cytokines and Gene Expression

Nworu and Akah (2015), showed anti-inflammatory activities of *Arctium lappa*, *Camellia sinensis*, *Echinacea angustifolia*, *Eleutherococcus senticosus*, *Panax ginseng*, and *Vaccinium myrtillus*. All the extracts depressed *IL1*β expression, but upregulated *NF*κ*B,* and *TNF*α were downregulated. Also the inhibitory actions of *Scrophularia striata* on pro-inflammatory mediators (IL-1β, TNF-α, and PGE2) was evaluated by Azadmehr *et al.,* (2012).

2.3.0 Oxidative Stress and Inflammation

Oxidative stress arise from the actions free radicals and reactive metabolites in the organism. (Durakova, 2010). At normal physiological state the reactive species are not a threat to the body because the body can remove them. There is a certain limit at which the body require the ROS/RNS because they are responsible for the manifestation of cellular functions including signal transductionpathways, defense against invading microorganisms and gene expression to the promotion of growth or death (Li, 2015).

Oxidative stress and inflammation are attributed with the development of many diseases such cardiovascular diseases, diabetes, and cancer. Reactive oxygen and Nitrogen species are released by inflammatory cells which leads to development of oxidative stress. The inflammatory cells after they are being activated they cause release of enzymes (neutral proteases, elastase, collagenase, acid hydrolases phosphatases, lipases, etc.), reactive species (superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid, etc.) and chemical mediators (eicosanoids, complement components, cytokines, chemokines, nitric oxide, etc.) which cause tissue damage and oxidative stress (Biswas, 2016). Production of large number of ROS/RNS leads to formationof proinflammatory cytokines. Also the activation of nuclear factor-kappa B (NF- κ B) and production of tumor necrosis factor-alpha (TNF- α) play a significant role in inflammatory processes and development of chronic diseases (Hussain, 2016).

2.3.1 Lipid Peroxidation

Lipid peroxidation is a chain reaction that lead to oxidation of unsaturated fatty acids. Free radical chain reaction is due to the removal of hydrogen from fatty acid molecule to form a lipid radicals (L_) which react immediately with oxygen, to forma LOO_, which attacks another lipid and

removes a hydrogen atom from it, resulting in the formation of lipid hydroperoxide (lipid peroxide; LOOH) and LOO_, which attacks another lipid to generate lipid peroxide, so lipid peroxide accumulates as the chain reaction proceeds (Yoshikawa and Naito, 2002).

The free radical peroxidation of lipids is an important factor in local injury to cell membranes and impairment of the activity of enzymes and receptors bound to the membrane, and the lipid peroxide thus produced can affect even remote organs (Yoshikawa and Naito, 2002).

2.3.2 Antioxidant System

Antioxidants are chemical compounds which contain monohydroxy/polyhydroxy phenol; they just work to slow down the lipid peroxidation. These compounds have low activation energy to donate hydrogen atom and therefore, cannot initiate the second free radicals. The free radical electrons are stable and thus, slow down the oxidation. Cells contain many antioxidant systems to prevent injury. Prevention of excessive ROS and repair of cellular damage is essential for cell's life (Noori, 2012).

Classification of Antioxidants

Antioxidants may be enzymatic or non-enzymatic. Enzymatic system directly/indirectly contributes to defense against the ROS. Catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, thioredoxin exhibits biological value. The non-enzymatic antioxidants are actually the scavangers of ROS and RNS; these involve glutathione, vitamin E and C (inhibits oxidation of membrane lipid), uric acid is the scavenger of peroxynitrite in plasma, albumin, bilirubin, N-Acetylcysteine (NAC), melatonin which directly reacts with ROS and form disulfides (Noori, 2012).

Endogenous Antioxidants

It can be categorized into primary antioxidants and secondary antioxidants. SOD, Catalase and Glutathione peroxidase are the primary antioxidant enzymes which inactivate the ROS into intermediates Besides the antioxidant enzymes, primary antioxidants are water soluble and lipid soluble. Ascorbate, glutathione, uric acid etc. are water soluble, and lipids soluble are tocopherols, ubiquinols and carotenoids, etc. Secondary antioxidant enzymes are Glutathione reductase, Glucose-6-Phosphate dehydrogenase, glutathione-S-transferase and ubiquinone work directly to detoxify ROS by decreasing the peroxides level and continuously supplying the NADPH and glutathione for primary antioxidant enzymes to maintain their proper functioning. Copper, iron, manganese, zinc, selenium enhances the antioxidant enzyme activities (Noori, 2012).

Exogenous antioxidants

These are mainly derived from food and other dietary sources. Several herbs, spices, vitamins, foods, vegetables etc exhibits antioxidant activities. Therefore, antioxidants based drugs for the treatment of various pathological diseases have gained attraction in clinical as well as research areas. Flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, isocatechins, epicatechin, etc found in natural foods are called phytochemicals. Numerous types of bioactive compounds are being used in clinical and preclinical trials from plant sources. Plant derived drugs medicinally useful as it contains terpenoid, alkaloids, glycosides, polyphenolics, and steroids (Noori, 2012).

2.4.0 Carrageenan-Induced Paw Oedema

Carrageenan is a generic name of a family of gel-forming and viscosifying polysaccharides. They are produced by extraction of some species of red seaweeds. Their main class is Rhodophyceae, and the specie name is Chondrus crispus. They are native to some parts of Europe and north America.

Most of its uses is in gelling, thickening, and as emulsifying agents. They also have medical and pharmaceutical applications especially in research on anti-inflammatory agent. Chemically carrageenan is a sulfated polygalactan with 15% to 40% of ester-sulfate content. Its relative molecular mass is about 100 kDa. It has an alternate units of d-galactose and 3,6-anhydro-galactoose, joined by α-1,3 and β-1,4-glycosidic linkage (Necas, and Bartosikova, 2013).

Carrageenan-induced paw oedema is the most widely used method for anti-inflammatory study. It involves administering an immunomodulatory substances, such as carrageenan, ovalbumin, or formalin into a right hind paw of the animals. But in the case of mouse part of ear is also used.

A solution of 1-3% carrageenan in saline in a doses of 50–150 µl. The development of oedema by carrageenan injection is biphasic, which also depend on the age and weight of the animal. In the early phase of carrageenan paw oedema the main mediators that play role include histamine, serotonin and bradykinin, and it is not inhibited by NSAIDs. The second accelerating phase is caused by induction of inducible cyclooxygenase (COX-2), but this phase is inhibited by NSAIDs. Prostaglandins release at the late phase is responsible for increased vascular permeability. The release of nitric oxide (NO), by nitric oxide synthase is also one of the mediators of acute inflammatory response. The early inflammatory response lasts for 6 h. The maximum duration for the second late response is about 72 h, and decline at about 96 h (Posadas *etal.,* 2004)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment and Chemicals

Distilled Water, Digital Weighing Machine, Digital Vernier caliper, Cages, Syringes (5mls, 2mls and 1ml), Spatula, Set of Dissecting Kit, Normal Saline, Ketamin hydrochloride, Diclofenac,

Containers, Pins, Carrageenan (purchased from Sigma Chemical Company St. Louis USA), IL-6 Elisa kits (Purchased at Wuhan Fine Biotech China).All otherdrugs and reagents were obtained commercially and are of analytical grades.

3.1.2 Plant Collection and Authentication

Leaves of *Syzigium guineense* were obtained from Duwo village of Darazo Local Government, Bauchi State in November, 2017. This plant was authenticated and given a voucher number (V/N: 053), by NamadiSanusi, from Botany unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

3.1.3 Animals

A total of thirty (30) adult male wistar rats weighing (100 – 150) grams were used for the study. They were obtained from National Veterinary Research Institute, VOM, Plateau state, Nigeria. The animals were housed in plastic cages under standard laboratory conditions with free access to food and water. Animals were allowed for two weeks to acclimatization to the laboratory environment before the commencement of the experiments. The experiment was carried out in the Pharmacology animal house, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. Ethical approval was obtained from Ahmadu Bello University Committee on Animal Use and Care with the approval number of ABUCAUC/2021/033.

3.2 Methodology

3.2.1 Extraction of Plant Materials

The leaves of *Syzygium guineense* was carefully cleaned with distilled water, then dried in the open air for five days. It wasgrounded to a coarse powder and passed through a sieve to obtain a fine powder. The dried powder was kept in an air-tight container. 120g powder was soaked in 100ml of distilled water. The mixture was shaken at intervals for 6 hours and then left to soak for 24 hours in a water bath set at 40 °C, followed by filtration. The filtrates obtained were concentrated to dryness at 40 °C under reduced pressure. The percentage yield obtained was 65 (w/w %) of aqueous extract, and the dried extract was stored in an air-tight container at 4 °C until use.

3.2.2 Phytochemical Analysis of Aqueous Leaf Extracts *Syzygium guineense*

The extracts were screened for the presence of different phytochemical constituents based on the following tests according to the methods of Tease and Evans (2002).

Test for Alkaloids

Dragendoff's Test: To 100mg of the extract, 1 ml of Dragendoff's reagent was added. A reddish brown precipitate indicates the presence of alkaloids (Tease and Evans, 2002).

Test for Anthraquinones

Bontrager's Test: to a 100mg of the extract in a dry test tube, 5ml of chloroform was added and was shaken for at least 5 minutes. This was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. Bright pink colour in the aqueous (upper) layer indicates the presence of free anthraquinones(Tease and Evans, 2002).

Test for Carbohydrates

Molisch Test: To 500mg of the extract in a test tube, few drops of molisch reagent was added and concentrated sulphuric acid was added down the side of the test tube to form a lower layer, a reddish colored ring at the interphase indicates the presence of Carbohydrates(Tease and Evans, 2002).

Test for Cardiac Glycosides

Ferric Chloride Test: To a 500mg of the extract, 5ml of dilute sulphuric acid was added and boiled on water bath for 10-15 minutes. This was then cooled and neutralized with 20% potassiun hydroxide solution. About 3ml of Ferric chloride solution was added. A green to blue colour will be produced because of the release of phenolic aglycones due the hydrolysis(Tease and Evans, 2002).

Test for Flavonoids

Shinoda Test: 200mg of the extract was dissolved in 1-2ml of 50% methanol in the heat Metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red colour indicates the presence of flavonoids (Tease and Evans, 2002).

Test for Saponins

Frothing Test: About 10ml of distilled water was added to 1000mg of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 minutes. A honeycomb froth that persists for 10-15 minutes indicates the presence of saponins (Tease and Evans, 2002).

Test for Tannins

Ferric Chloride Test: To 1000mg of the extract, 3-5 drops of ferric chloride solution was added. A greenish-black precipitate indicates the presence of condensed tannins, while hydrolysable tannins give a blue or brownish-blue precipitate (Tease and Evans, 2002).

Test for Triterpenes

Lieberman Bucchard Test: To 100mg of the extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Colour changes were observed immediately and over a period of one hour, blue-green colour in the upper layer and areddish, pink or purple colour indicates the presence of triterpene (Tease and Evans, 2002).

3.2.3Acute Toxicity (LD_{50}) Study

Acute toxicity test in rats was carried out in two phases according to the method of Lorke (1983). In the first phase, three groups of three rats each were given orally, 10, 100 and 1000 mg/ kg of body weight of the aqueous extract *Syzygium guineense*respectively and monitored for 24 h for physical signs of toxicity and mortality. The rats were subsequently observed for two weeks for delayed signs of toxicity and/or mortality. In the second phase, three groups of one rat each wereorally administered with 1600, 2900 and 5000 mg/kg respectively and monitored likewise.

3.2.4Experimental Design

Thirty albino wistar rats were used for the study, the animals were divided into six (6) groups of five (5) rats each. All administration were done orally one hour before the induction of inflammation and the animals were grouped as follows:

Group I: Normal Saline (1mg/kg)

Group II: 0.1 ml of 0.1% Carrageenan.

Group III:0.1 ml of 0.1% Carrageenan + Diclofenac (10mg/kg) (Aurelie, 2017)

Group IV: 0.1 ml of 0.1% Carrageenan + SG (250mg/kg)

Group V : 0.1 ml of 0.1% Carrageenan+ SG (500mg/kg)

Group VI 0.1 ml of 0.1% Carrageenan+ SG (1000mg/kg

3.2.5Test For Anti Inflammatory Activity

Rats were administered with SG orally at doses of 250, 500, 1000mg/kg and Diclofenac 10mg/kg. One hour after the administrations, each rat was injected with 0.1ml of carragenan (1%w/v) into the subplanter region of the right hind paw. The thickness of the paw wasmeasured every hour from 0h to 5h after Carrageenan injection by using a digital Vernier calliper.

$$\text{Percentage inhibition} = \frac{[Ct-C0]CGN - [Ct-C0]test \times 100}{[Ct-C0]CGN} \quad \text{Equation (1)}$$

Where: Co = Mean paw size at 0 h after carrageenan injection, Ct = Mean paw size at 5h after carrageenan injection, CGN = Carragenan without treatment group (Winter *et al.*, 1962).

3.2.6 Collection of Blood Sample

At the end of treatment hours, the animals were anaesthesized by administering 10mg/kg ketamine hydrochloride. Blood sample was collected through cardiac puncture using 5ml syringes. About 2ml of blood was collected for differential white blood cell in EDTA bottles according to the method of Dacie and Lewis (1975). And 3ml was collected in a non-heparinized bottles and serum collected for IL-6, and CRP and oxidative stress bio-markers, biochemical assays.

3.2.6.1 Assessment of Serum Interleukin 6 (IL-6)

The serum collected from the above groups were subjected to IL-6 assay using enzyme- linked immunosorbent assay (ELISA) kits according to the recommended procedure (Wuhan Fine Biotech, China). The IL-6 levels were measured by pipetting 50 µL of sample and 100 µL of standard diluent buffer into the wells of a microtiter plate coated with an antibody specific to rat. After adding biotinconjugate to all the wells, the plate was incubated at 18-25°C for 2 h. Streptavidin peroxidase HRP was then added and incubated for 30 min to bind to the biotinylated antibody. After two more washings with assay buffer to remove unbound enzymes, color was developed by adding stabilized chromogen tetramethylbenzidine, and a stop solution. Finally, the

optical density was calculated with an Elisa microplate reader at 450 nm and the IL-6 was quantified by comparing the sample to the standard curve generated from the kit. The results were expressed as cytokine concentrations (pg/mL).

3.2.6.2 Assessment of Serum C-Reactive Protein (CRP)

Before the test was conducted the reagents and samples were allowed to reach room temperature as the sensitivity of the test might reduced with low temperatures. 50µL of the sample and one drop of each positive and negative controls were placed in separate circles on the slide test. The CRP latex was mixed vigorously and one drop of it was added next to the samples to be tested. The drops were mixed with the stirrer. The results were recorded after two minutes. The presence of agglutination indicates CRP concentration equal or greater than 6mg/L.

Calculations: The approximate CRP concentration in the sample is calculated as:

6×CRP titre = mg/L - -- - - - - - - - - -- - - - -- - - - - -- - - - - -- -- - Equation (2)

(Yamamoto *et al.*, 1993).

3.2.6.3Differential White Blood Cells Counts

Differential White blood cell counts was based on method of Dacie and Lewis (1975). A thin blood smear were made by placing the spreader at angleof about 45^{o}. The blood smears were allowed to dry, and then flooded with Leishman's stain. After allowing it to stand for 3 minutes, it was then diluted with buffered distilled water and allowed to stain further for 7 minutes. It was then washed with buffered water twice, and allowed to dry. The cells were examined under the microscope with oil immersion objective. The cells were counted systematically, and expressed in percentage.

3.2.7Assessment of Biomarkers of Oxidative Stress

3.2.7.1Lipid peroxidation (MDA)

Lipid peroxidation was measured by the modified method of Niehau and Samuelson (1968) as described by Akanji *et al.* (2009). 150µl of sample was treated with 2ml of TBA-TCA-HCL reagent (1:1:1 ratio) and place in a water bath at 90^{o}C for 60 minute, the mixture was cooled and centrifuged at 3000 rpm for 5minute and the absorbance of the pink supernatant (TBA-Malonaldehyde complex was then measured at 535nm. Malonaldehyde formed was then calculated using the Molar extinction coefficient of 1.56x 10^{-5} $cm^{-1}M^{-1}$.

TBARS Conc. (nmol/mg protein) = {Absorbance of sample / 1.56x10^{-5} x protein Conc. (mg/ml)}---------- Equation (3)

(Niehau and Samuelson, 1968)

3.2.7.2 Catalase activity (CAT)

Catalase (CAT) activity was measured using Abebi's method (1974). Exactly 100µl of sample was added to a test tube containing 2.80ml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 1ml of freshly prepared 30mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240nm for 5 minute on a spectrophotometer. A Molar extinction coefficient (E) of 0.041mM^{-1} -cm^{-1} was used to calculate the Catalase activity.

3.2.7.3 Reduced glutathione concentration (GSH)

Reduced glutathione (GSH) concentration measurement was done according to Ellman (1959) as describe by Rajagopalan *et al.* (2004).

To 150µl of sample (in phosphate - saline buffer pH 7.4), 1.5ml of 10% TCA was added and centrifuge at 1500rpm for 5 minute. 1 ml of the supernatant was treated with 0.5ml of Ellman's

reagent and 3ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm.The quantity of GSH was obtained from the graph of the GSH standard curve.

3.2.7.4 Superoxide dismutase(SOD)

Superoxide dismutase (SOD) was determine by the method describe by Fridovich (1989). Sample of 0.1ml was diluted in 0.9ml of distilled water to make 1:10 dilution of micro some. An aliquant mixture of 0.2ml of the diluted micro some was added to 2.5ml of 0.05M carbonate buffer. The reaction was started with the addition of 0.3ml of 0.3mM Adrenaline. The reference mixture contained 2.5ml of 0.05M carbonate buffer, 0.3ml of 0.3mM Adrenaline and 0.2ml of distilled water. The Absorbance was measured over 30 seconds up to 150 seconds at 480nm.

Calculations:

Increase in absorbance per minute = $(A_2 - A_1)/2.5$ -- -- - -- - - - - -- - -- - - - -- - Equation (4)

% Inhibition = 100 - {(Increase in absorbance for sample/Increase in absorbance of blank) x 100}(Fridovich, 1989).

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of Adrenaline to adenochrome in 1 minute.

3.3Statistical Analysis

All data obtained were presented as Mean ± standard error of mean (SEM), and data was analysed using one way analysis of variance (ANOVA) followed by*Tukey's post hoc* test using SPSS (version 23). Values of $p \leq 0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Acute toxicity (LD_{50}) of the Aqueous Leaf Extract*Syzygium guineense*

Table 4.1 showed the result of the acute toxicity test of the aqueous extract of *Syzygium guineense.* In stage one of the test no signs of toxicity and mortality were recorded in the animals of each groups. Also there were no signs of toxicity and mortality observed in the animals after undergoing the second phase of the test. The LD_{50} of Aqueous Exracts *Syzygium guineense*was determined to be greater than 5000 mg/kg.

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Table 4.1 Acute toxicity (LD_{50}) of Aqueous Extracts of *Syzygium guineense*

	Dose (mg/kg)	Mortality
Phase One	10	0/3
	100	0/3
	1000	0/3
Phase Two	1600	0/1
	2900	0/1
	5000	0/1

LD_{50}>5000 mg/kg

4.2 Phytochemical screening of the Aqueous Leaf Extract of *Syzygium guineense*

Table 4.2 shows the phytochemical constituents of *Syzygium guineense*, after conducting various tests. The constituents include carbohydrates, Cardiac glycosides, Flavonoids, Saponins, Steroids, Tannins and Triterpenes. The alkaloids and anthraquinones were absent in aqueous leaf extract of *Syzygium guineense.*

Table 4.2: Phytochemical constituents of the Aqueous Leaf Extract of *Syzygium guineense*

Constituents	**Inferences**
Alkaloids	-
Anthraquinones	-
Carbohydrates	+
Cardiac glycosides	+
Flavonoids	+
Saponins	+
Steroids	+
Tannins	+
Triterpenes	+

+ Present

- Absent

4.3 INFLAMMATORY PARAMETERS

4.3.1 Effectof Aqueous Leaf Extract of*Syzygium guineense* on Acute Oedema of Rat Hind Paw

Table 4.3 shows the effects of various doses of aqueous leaf extract of *Syzygium guineense* (250mg/kg, 500mg/kg, 1000mg/kg) at different time intervals after carrageenan administration. After 0h and 1h only 1000mg/kg showed statistically significantly (P≤ 0.05) increase in paw thickness (2.2+0.1mm) compared to normal saline (2.4+0.0 mm). After 2h, all the three doses including the standard drug showed statistically significantly (P≤ 0.05) higher paw thickness (2.5±0.0, 2.6+0.0, and 2.6+0.0 mm) compared to normal saline (2.0±0.0 mm). After 3h all the doses of *Syzygium guineense* showed statistically significantly (P≤ 0.05) lower paw thickness (2.4±0.1, 2.4+0.0 and 2.5+0.0 mm) compared to carrageenan group (3.1±1.2 mm). After 4h all the three doses of *Syzygium guineense* showed statistically significantly (P≤ 0.05) lower paw thickness

(2.3±0.1, 2.4+0.0and 2.4+0.0 mm) compared to carrageenan (2.9±0.1 mm). After 5h all three doses of *Syzygium guineense* showed statistically significantly (P≤0.05) lower thickness compared to (2.3±0.1, 2.3+0.0, 2.3+0.0 mm) carrageenan (2.9±0.2 mm). The diclofenac group recorded highest (89.0%) percentage inhibition of oedema, followed by 1000mg/kg dose which recorded 84.6%. The least percentage inhibition was 59.3% recorded by 250mg/kg dose.

Table 4.3 Effect of Aqueous Leaf Extract of *Syzigium guineense* on Acute Oedema of Hind Paw of Wistar Rats

	Paw Thickness (mm)						% Inhibition
Group	**0h**	**1h**	**2h**	**3h**	**4h**	**5h**	**%**
1: NS	2.0±0.0	2.0±0.0	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b,c}	2.0±0.0^{b}	100.0
2: CGN	1.9±0.1	2.3±0.1	2.6±0.0^{a}	3.1±1.2^{a,c}	2.9±0.1^{a,c}	2.9±0.2^{a,}	0.00
3: DIC+CGN	2.1±0.0	2.2±0.0	2.3±0.1^{a}	2.3±0.0^{b}	2.4±0.0^{a,b}	2.2±0.0^{b}	89.0
4: SG1+CGN	1.9±0.1	2.3±0.1	2.5±0.0^{a}	2.4±0.1^{b}	2.3±0.1^{b}	2.3±0.1^{b}	59.3
5: SG2+CGN	2.1±0.1	2.3+0.0	2.6+0.0^{a}	2.4+0.0^{b}	2.4+0.0^{b}	2.3+0.0^{b}	78.0
6:SG3+CGN	2.2+0.1^{a}	2.4+0.0^{a}	2.6+0.0^{a}	2.5+0.0^{a,b}	2.4+0.0^{a,b}	2.3+0.0^{b}	84.6

Values having different Superscript, a, b,c are statistically significant (P≤0.05).. NS: Normal Saline, CGN: Carrageenan group, DIC: 10 mg/kg ofDiclofenac, SG1: 250mg/kg of *Syzygium guineense*, SG2: 500mg/kg of *Syzygium guineense*, SG3: 1000mg/kg of *Syzygium guineense.* a= Significant at (P≤0.05) when compared to Normal Saline, b= Significant at (P≤0.05) when

compared to 0.1% Carrageenan, c= Significant at (P≤0.05) when ,compared to Standard drug, Diclofenac.

4.3.2Serum IL-6 levels

Figure 4.1 showed the effect of aqueous leaf extract of *Syzygium guineense* on serum IL-6 levels of adult male wistar rats. IL-6 levels of all the groups: CGN (816.23±54.84 Pg/ml), Dic 10mg/kg (373.27±21.59 Pg/ml), SG 250mg/kg (765.07±20.74 Pg/ml), SG 500mg/kg (679.35±26.88 Pg/ml), and SG 1000mg/kg (691.26±50.66 Pg/ml) were significantly higher (P≤0.05) compared to the normal saline group (182.57±38.20 Pg/ml). The Diclofenac group was significantly (P≤0.05) lower (373.27±21.59 Pg/ml) when compared to carrageenan group (816.23±54.84 Pg/ml). All the treatment groupsSG 250mg/kg (765.07±20.74 Pg/ml), SG 500mg/kg (679.35±26.88 Pg/ml), and SG 1000mg/kg (691.26±50.66 Pg/ml) were significantly (P≤0.05) higher compared to diclofenac group (373.27±21.59 Pg/ml). The groups treated with *Syzygium guineense* did not show statistically (P≤0.05) significantly lower serum IL-6 level, when compared to carrageenan group.

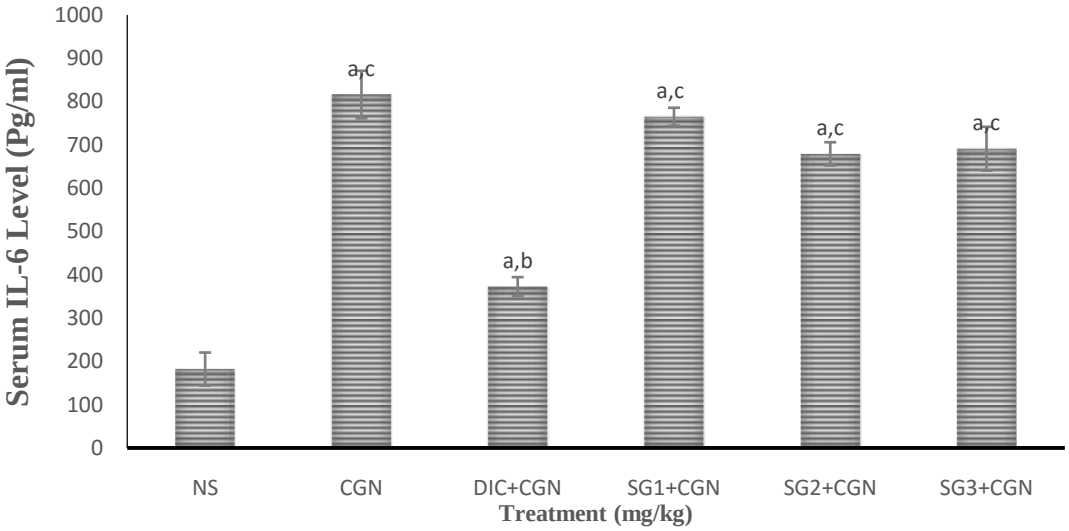

Figure 4.1: Effect of Aqueous Leaf Extract of *Syzygium guineense* on serum IL-6 level of Adult Male Wistar Rats.

Values having different Superscript, a, b,c are statistically significant (P≤0.05).. NS: Normal Saline, CGN: Carrageenan group, DIC: 10 mg/kg of Diclofenac, SG1: 250mg/kg of *Syzygium guineense*, SG2: 500mg/kg of *Syzygium guineense*, SG3: 1000mg/kg of *Syzygium guineense*. a= Significant at (P≤0.05) when compared to Normal Saline, b= Significant at (P≤0.05) when compared to 0.1% Carrageenan,c= Significant at (P≤0.05) when compared to Standard drug, Diclofenac.

4.3.3 Serum CRP Levels

Figure 4.2 shows the effects of aqueous leaf extract of *Syzygium guineense* (250mg/kg, 500mg/kg and 1000mg/kg) on serum CRP levels of adult male wistar rats. All the three doses did not show statistical significant ($P \leq 0.05$) decrease in serum CRP levels compared to normal saline, carrageenan and diclofenac groups.

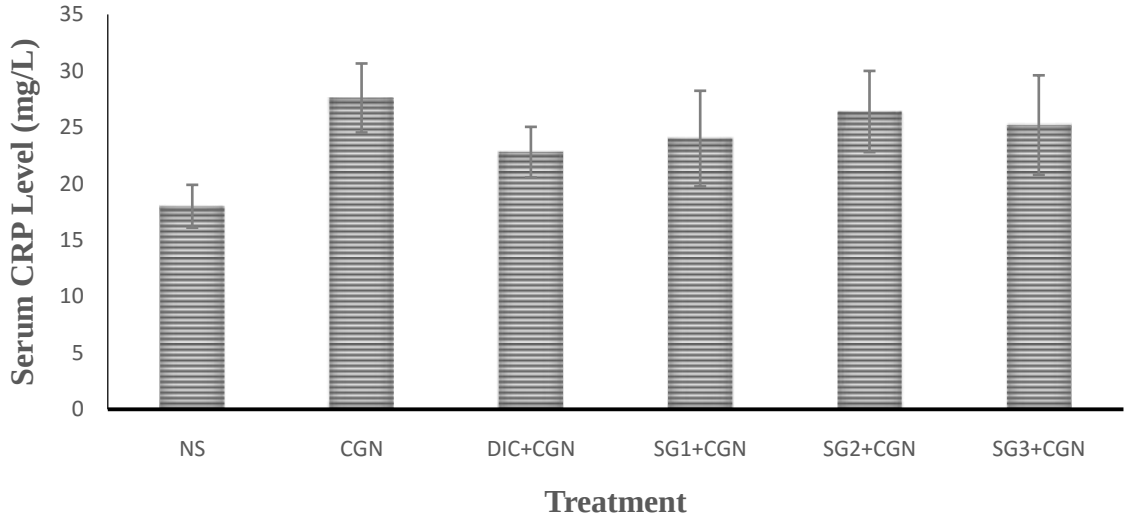

FIGURE 4.2: **Effects of Aqueous Leaf Extract of *Syzygium guineense* on Serum CRP level of Adult Male Wistar Rats**.

NS: Normal Saline, CGN: Carrageenan group, DIC: 10 mg/kg of Diclofenac, SG1: 250mg/kg of *Syzygium guineense*, SG2: 500mg/kg of *Syzygium guineense*, SG3: 1000mg/kg of *Syzygium guineense*. a= Significant at (P≤0.05) when compared to Normal Saline, b= Significant at (P≤0.05) when compared to 0.1% Carrageenan, c= Significant at (P≤0.05) when ,compared to Standard drug, Diclofenac.

4.3.4 : Effects of Aqueous Leaf Extract of *Syzygium guineense* on Differential WBC count of Adult Male Wistar rats

Table 4.4 shows the effect of aqueous leaf extract of *Syzygium guineense* (250mg/kg, 500mg/kg, 1000mg/kg) on differential WBC count of adult male wistar rats. 250mg/kg dose of *Syzygium guineense* showed statistically significantly ($P \leq 0.05$) higher Neutrophil counts (35.20+1.59) compared to normal saline (28.80+0.58) and carrageenan groups (28.80+1.39). The 250mg/kg dose also caused a statistically significantly ($P \leq 0.05$) lower Basophils levels (0.00+0.00) compared to diclofenac (1.20+0.20). The 1000mg/kg dose of *Syzygium guineense* showed a statistically significantly ($P \leq 0.05$) higher Eosinophil levels (2.00+0.31) compared to normal saline (0.00+0.00) and carrageenan groups (0.00+0.00). No statistical significant ($P \leq 0.05$) difference in Lymphocytes counts among the all groups. Only the 250mg/kg dose significantly ($P \leq 0.05$) decreased the monocyte count (4.60+1.12) compared to normal saline (8.60+1.44) and carrageenan groups (9.20+0.73).

Table 4.4: Effect of Aqueous Leaf Extract of *Syzygium guineense* on Differential White Blood Cells Count of Adult Male Wistar Rats

	Differential White Blood Cell Count				
Group	**N (%)**	**B (%)**	**E (%)**	**L (%)**	**M (%)**
NS	28.08+0.58	0.00+0.00	0.00+0.00[c]	62.40+1.54	8.60+1.44
CGN	28.80+1.39	0.20+0.10	0.00+0.00[c]	62.20+0.97	9.20+0.73
DIC+CGN	29.40+0.40	1.20+0.20[a,b]	2.80+0.37[a,b]	58.40+1.03	8.20+1.83
SG1+CGN	35.20+1.59[a,b]	0.00+0.00[c]	0.00+0.00[c]	59.00+1.00	4.60+1.12[a,b,c]
SG2+CGN	25.80+2.58	0.40+0.24	0.00+ 0.00[c]	59.40+1.17[c]	8.0+1.26
SG3+CGN	30.00+0.63	0.80+0.20	2.00+0.31[a,b]	61.20+0.67	7.80+1.39

Values having different Superscript, a, b,c are statistically significant (P≤0.05). NS: Normal Saline, CGN: Carrageenan group, DIC: 10 mg/kg of Diclofenac, SG1: 250mg/kg of*Syzygium guineense*, SG2: 500mg/kg of *Syzygium guineense*, SG3: 1000mg/kg of *Syzygium guineense.* a= Significant at (P≤0.05) when compared to b= Significant at (P≤0.05) when compared to 0.1% Carrageenan, c= Significant at (P≤0.05) when ,compared to Standard drug, Diclofenac.

4.4 Lipid Peroxidation and Biomarkers of Oxidative Stress

4.4.1 Serum MDA levels

Figure 4.3 shows the effects of aqueous leaf extract of *Syzygium guineense* (250mg/kg, 500mg/kg and 1000mg/kg) on serum MDA levels of adult male wistar rats. There was no statistical significant ($p<0.05$) difference in serum MDA levels between the three doses of *Syzygium guineense* and normal saline and carrageenan groups.

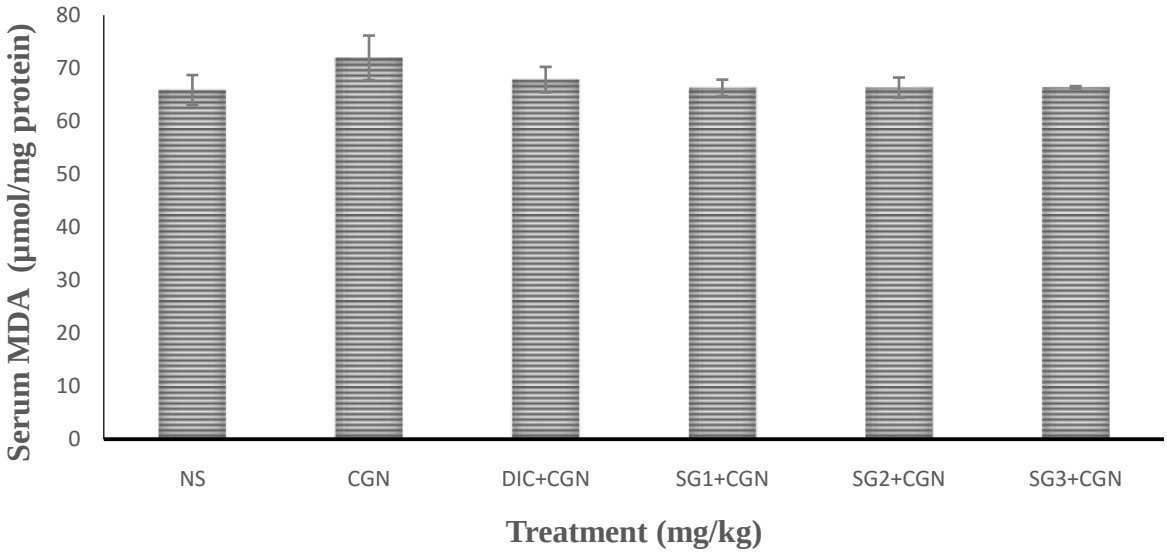

Figure 4.3: Effect of Aqueous Leaf Extract of *Syzygium guineense* on serum MDA of adult male wistar rats.

NS: Normal Saline, CGN: Carrageenan group, DIC: 10 mg/kg of Diclofenac, SG2: 250mg/kg of*Syzygium guineense*, SG2: 500mg/kg of *Syzygium guineense*, SG3: 1000mg/kg of *Syzygium guineense*.

4.4.2 Serum CAT Levels

Figure 4.4 shows the effects of*Syzygium guineense* on serum Catalase level of adult male Wistar rats. There was statistically significant increase ($P \leq 0.05$) in Catalase levels by Carrageenan (18.58±0.55 U/mg protein), Diclofenac (18.54±1.23 U/mg protein) and 250mg/kg dose (20.44±1.00 U/mg protein) of *syzygium guineense*compared to normal saline (15.12±0.36 U/mg protein). None of the doses of *Syzygium guineense* showed statistically significantly ($p<0.005$) higher serum Catalase level compared to carrageenan group.

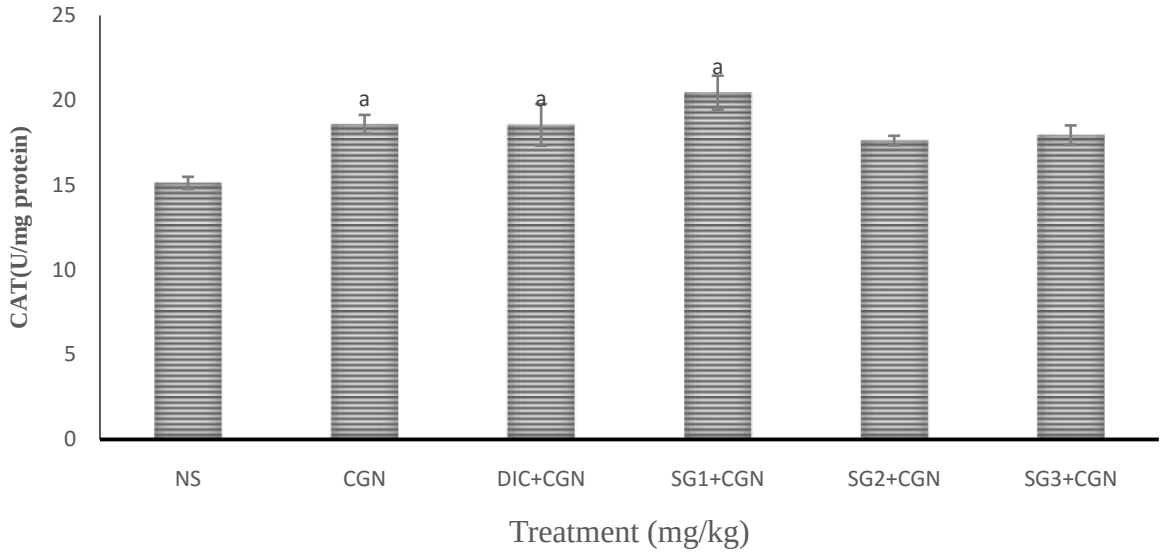

Figure 4.4Effect of Aqueous Leaf Extract of *Syzygium guineense* on Serum CAT of Adult Male Wistar Rats.

NS: Normal Saline, CGN: Carrageenan group, DIC: 10 mg/kg of Diclofenac, SG2: 250 mg/kg of *Syzygium guineense*, SG2: 500 mg/kg of *Syzygium guineense*, SG3: 1000 mg/kg of *Syzygium guineense*. a= Significant at ($P \leq 0.05$) when compared to Normal Saline.

4.4.3 Serum SOD Levels

Only 250mg/kg (29.00±1.7 U/ml) of the extract show statistical significant (P≤0.05) increase in serum SOD level compared to normal saline (26.50±0.34 U/ml). Other doses of *Syzygium guineense* did not show statistical significant (P≤0.05) difference in serum SOD levels when compared to normal saline, carrageenan and diclofenac groups.

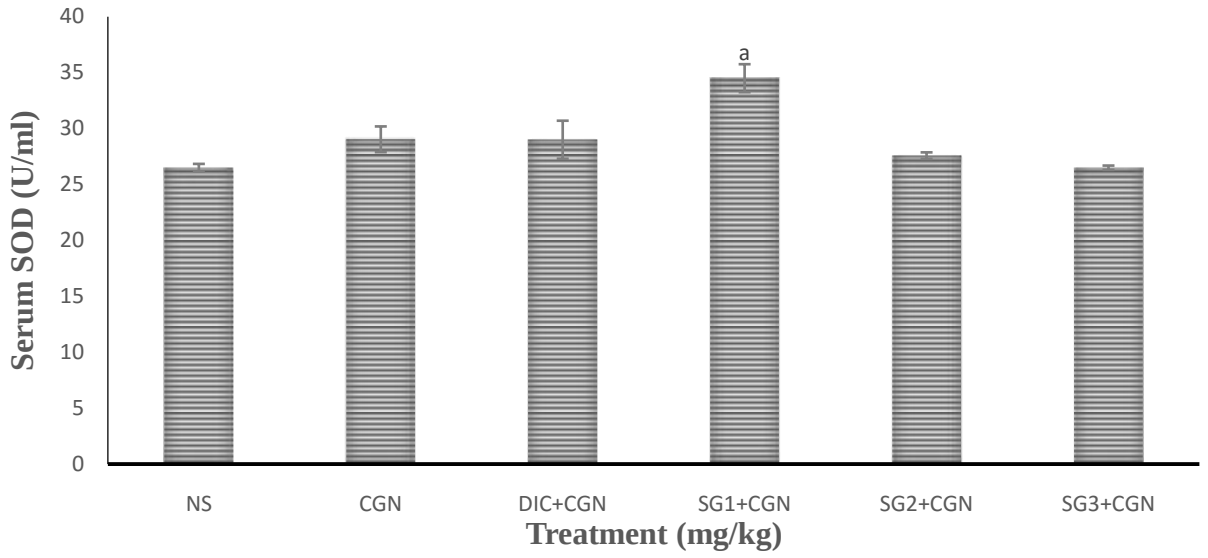

Figure 4.5: Effect of Aqueous Leaf Extract of *Syzygium guineense* on Serum SOD of adult male Wistar rats.

NS: Normal Saline, CGN: Carrageenan group, DIC: 10 mg/kg of Diclofenac, SG2: 250 mg/kg of *Syzygium guineense*, SG2: 500 mg/kg of *Syzygium guineense*, SG3: 1000 mg/kg of *Syzygium guineense*. a= Significant at ($P \leq 0.05$) when compared to Normal Saline.

4.4.4 Serum GSH Levels

Figure 4.6 shows the effects of aqueous leaf extract of *Syzygium guineense* (250mg/kg, 500mg/kg and 1000mg/kg) on serum GSH level. All the three doses of*Syzygium guineense*, did not show statistically significantly ($P \leq 0.05$) higher serum GSH levels when compared to normal saline, and carrageenan groups. The standard drug, diclofenac (40.04±2.97µg/ml) showed statistically significantly ($P \leq 0.05$) higher serum GSH levels compared to normal saline (27.82±0.97µg/ml), carrageenan (29.76±0.47µg/ml) and *Syzygium guineense* groups.

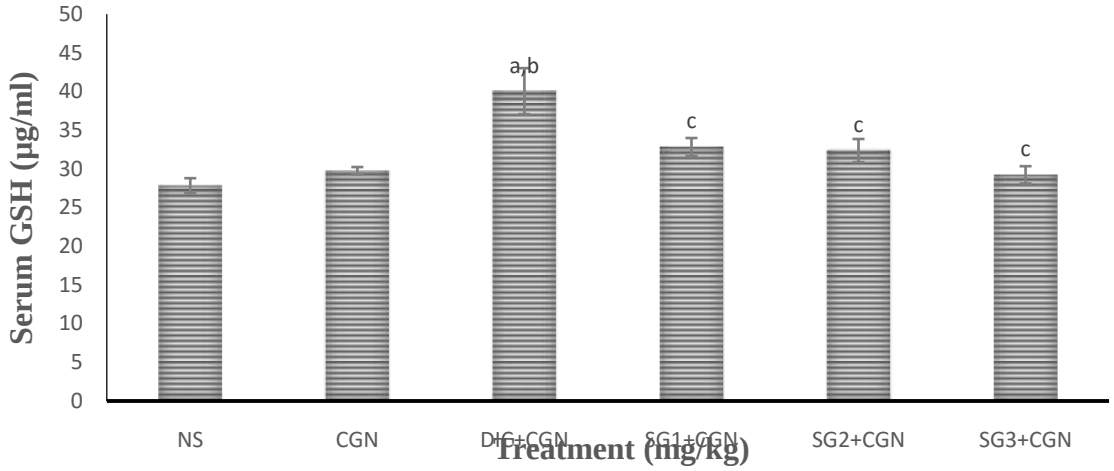

Figure 4.6: Effect of Aqueous Leaf Extract of *Syzygium guineense* on serum GSH of adult male wistar rats.

Values having different Superscript, a, b,c are statistically significant (P≤0.05). NS: Normal Saline, CGN: Carrageenan group, DIC: 10 mg/kg of Diclofenac, SG2: 250 mg/kg of *Syzygium guineense*, SG2: 500 mg/kg of *Syzygium guineense,* SG3: 1000 mg/kg of *Syzygium guineense*. a= Significant at (P≤0.05) when compared to Normal Saline, b= Significant at (P≤0.05) when compared to 0.1% Carrageenan, c= Significant at (P≤0.05) when ,compared to Standard drug, Diclofenac.

CHAPTER FIVE

5.0 DISCUSSION

The aqueous leafextract of *Syzygium guineense*was evaluated for acute toxicity, phytochenical screening and ant-inflammatory properties. Acute toxicity studies was conducted to determine the safety of the plant based on the method of Lorke (1983).The aqueous leaf extract of *Syzygium guineense* did not cause any sign of toxicity. The LD_{50} of the extract was found to be greater than 5000mg/kg body weight showing no signs of toxicity. According to De Oliveira *et al., (*2014), a pharmacological agent is considered safe if it does not cause signs of toxicity such as spontaneous motor activity, lack of appetite, nasal discharge, urination, diarrhea, convulsion and/or death. The result agreed with the finding of Lorato (2016) who determined the LD_{50} of hydromethanolic extract of *Syzygium guineense*to be greater than 5000mg/kg. Ior *et al.,* (2012), found the intraperitoneal LD_{50}ethanolic extract of the leaf of *Syzygium guineense* to be 3.807g/kg. On the other hand the toxicity of the plant material seems dependent on the active metabolites, concentration administered, rate of their conversion in the liver and their consequent excretion(Adam *et al.,* 2013).

The phytochemical studies was also conducted according to the methods of (Tease and Evans, 2002), which revealed the presence of active molecules such carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and triterpenes.

Carrageenan induced rat paw oedema is the most widely used method to determine the anti-inflammatory effect of a particular drugs. Some of the advantages of the method include simplicity,

rapid induction of symptoms, and reproducibility. Diclofenac was chosen for comparison in this study because it is most commonly used (Aurelie *et al.*, 2017).

All the doses (250, 500 and 1000 mg/kg) of *Syzygium guineense* as well and the standard drugs showed statistically significant ($p \leq 0.005$) decrease in the thickness of rat hind paw after the three hours compared to control. This result agrees with the findings of Ior *et al.*, (2012), who showed that the methanolic extract of bark of *Syzygium guineense*significantly ($P \leq 0.05$) reduced the egg albumin induced inflammation in rats in a dose dependent manner two to three hours after the injection of inflammatory agent.

None of the doses (250, 500 and 1000 mg/kg) of aqueous leaf extract of *Syzygium guineense* significantly ($P \leq 0.05$) decreased IL-6 level when compared with carrageenan. Only the standard drug diclofenac significantly ($P \leq 0.05$) reduced the Serum IL-6 level.A possible reason behind the inability of the extract to significantly decrease the serum IL-6 level could be due to the presence of immune-modulatory carbohydrates as revealed by phytochemical analysis which cause further release of inflammatory mediators. This agreed with finding of Ghildyal *et al.,* (2010), who showed that the leaves of *Syzygium guineense* contain arabinogalactan or pectic polysaccharides which stimulate macrophages, dendritic cells and B cells to release cytokines pro-inflammatory cytokines IL-1, IL-6, and TNFα.

C-reactive protein is a protein which reacts with pneumococcus polysaccharide C in the serum of patients with an acute inflammation. Its physiological roles are the activation of classical complement pathway, mobilization and activation of leukocytes, stimulation of phagocytosis and

cytokine secretion by monocytes. There were no statistical significant (P≤0.05) difference in serum CRP levels between the *Syzygium guineense* treated groups, normal saline, carrageenan and diclofenac groups.The result disagree with the findings of Aurelie *etal.,* (2017), who demonstrated the decrease in CRP level after carrageenan administration in the right hind paw of male wistar rats.

In this study, there was statistically significant (P≤0.05) increase in neutrophil count by the lowest (250mg/kg) doses of *Syzygium guineense,* compared to normal saline and carrageenan groups. Only the 1000mg/kg dose of *Syzygium guineense*and diclofenac caused statistically significant (P≤0.05) increase in eosinophil counts compared to normal saline and carrageenan groups. Also there were no statistically significant (P≤0.05) change in lymphocyte count between the *Syzygium guineense* treated groups compared to normal saline, carrageenan and diclofenac groups. The lowest dose of *Syzygium guineense* caused statistically significant (P≤0.05) decrease in monocytes counts compared to all groups. In a similar study conducted by Lorato (2016), there were no observed statistically significant (P≤0.05) difference between treated groups and control. The observed increase in neutrophil and eosinophil counts by some doses of *Syzygium guineense*can be attributed to immune-modulatory effect of the extract (Ghildyal *et al.,* 2010). Liao *et al.,* (2013) did not found statistically significant (P≤0.05) difference between leucocytes counts of the treated groups and control group. Where there was decrease in leucocyte count was due to the fact that the extract might contain some bioactive agents that could cause destruction or impair production of WBCs or contain some components that reduced the production of these regulatory factors or interfere with the sensitivity of the committed stem cells responsible for the production of white blood cells. Increase in neutrophil and eosinophil count could also be due reaction of rats to foreign substances, which alter their normal physiological processes. It also indicates a stimulation of the immune

system which protects the rats against infection that might have been caused by chemical and secondary infections, or it could be attributed to an increase in leukocyte mobilization (Liao *et al.*, 2013).

There were no statistical significant ($P \leq 0.05$) difference between all the groups for serum MDA level. The antioxidant enzymes assayed in this study were CAT, SOD, and GSH. All the three doses of *Syzygium guineense* did not significantly ($P \leq 0.05$) increase the serum CAT level compared to carrageenan group. The lowest dose of the extract significantly ($P \leq 0.05$) increase the serum level of SOD when compared to normal saline group. All three doses of the extract did not show any statistically significant ($P \leq 0.05$) increase in the serum GSH levels when compared with carrageenan group. The antioxidant effect revealed by 250mg/kg dose of the extract agreed with findings of Pieme *et al.*, (2014), who showed that the antioxidant activity of *Syzygium guineense*, is related to its high phenolic content (phenols, flavonoids and flavonols). Antioxidant activity of polyphenols from natural origin occurs in various mechanisms such as the prevention of chain initiation, peroxide decomposition, trapping of free radicals, reducing ability, and the binding of transition metal ions. Phenolic compounds have the ability to transfer hydrogen atoms which are generally associated with the presence of reducing agents (Pieme *et al.*, 2014).

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CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

In the present study anti-inflammatory effects of aqueous leaf extract of *Syzygium guineense* was investigated in carrageenan-induced paw oedema of adult male wistar rats. The plant showed protective effects against inflammation, by lowering the thickness of right hand pawoedema. The serum levels of IL-6, CRP,MDA, and, were not significantly($P \leq 0.05$) affectedby *Syzygium guineense* extract. Also the serum levels of anti-oxidant enzymes CAT, and GSH were not significantly ($P \leq 0.05$)different. But the serum levels of SOD was significantly ($P \leq 0.05$) increased in the 250mg/kg group compared to normal saline group. There was statistical significant ($P \leq 0.05$) increase in Neutrophils (250mg/kg)Eosinophils (1000mg/kg) countsand decrease in monocytes (250mg/kg)counts compared to normal saline and carrageenan groups.

6.2 Conclussion

The acute toxicity studies characterized the aqueous leaf extract of *Syzygium guineense* to be not toxic. The aqueous leaf extract of 1000mg/kg dose of *Syzygium guineense* inhibited paw oedema by 84.6%. It also increased neutrophil and eosinophil counts but decreased the monocytes counts. The extract has very little anti-oxidatant properties. The effects produced by aqueous leaf extract of *Syzygium guineense* could be due to the presence of various phytochemicals such as flavonoids, phenols and carbohydrates.

6.3Recommendations

Based on the findings of this study, the following recommendation should be considered

1. Awareness should be created in the eyes of the public about the possible medicinal values of plants like *Syzygium guineense* in order to help in conservation of these plants.
2. The need to investigate the exact mechanism of action through which *Syzygium guineense* protects against inflammation.
3. There is a need for testing the *Syzygium guineense* on chronic inflammation and other models of inflammatory conditions.
4. That the different phytochemicals in the plant should be isolated and then be mixed in different proportion and tested against inflammatory conditions and associated disorders.

6.4 Contributions To Knowledge

1. Aqueous leaf extract of *Syzygium guineense*at all doses (250mg/kg, 500mg/kg, 1000mg/kg) inhibited in paw oedema with percentages inhibitions 59.3%, 78.0%, 84.6% respectively.

2. The 250mg dose of *Syzygium guineense* significantly ($P \leq 0.05$) increase the serum levels of anti-oxidant enzyme, SOD (34.50±1.25 U/ml), compared to normal saline group (26.50±0.34 U/ml).

3. The 250mg/kg and 1000mg/kg doses of *Syzygium guineense* significantly ($P \leq 0.05$) increases Neutrophils and Eosinophils counts with values 35.20±1.59 and 2.00±0.31 respectively. The monocytes counts was significantly ($P \leq 0.05$) decreased by 250mg/kg dose with values 4.60±1.12.

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LIST OF APPENDICES

8.1 Effect of *Syzygium guineense* on serum Interleukin-6 level of Adult Male Wistar Rats.

GROUP/TREATMENT	INTERLEUKIN 6
NS	182.57±38.20
CGN	816.23±54.84[a,c]
DIC	373.27±21.59[a,b]

SG1+CGN	765.07±20.74[a,c]
SG2+CGN	679.35±26.88[a,c]
SG3+CGN	691.26±50.66[a,c]

Values having different Superscript, a, b,c are statistically significant ($p \leq 0.05$). (One-way ANOVA followed by Tukey's Post Hoc Test). NS: Normal Saline, CGN: Carrageenan group, DIC: 10 mg/kg of Diclofenac, SG1: Group administered with 250mg/kg *Syzygium guineense*, SG2: Group administered with 500mg/kg of *Syzygium guineense*, SG3: Group administered with 1000mg/kg of *Syzygium guineense*.a= compared to Normal Saline, b= compared to 0.1% Carrageenan, c=compared to Standard drug, Diclofenac.

8.2 Effects of *Syzygium guineense* on Serum C Reactive Protein Level of Adult Male Wistar Rats

GROUP/TREATMENT	C Reactive Protein (mg/L)
NS	18.0±1.90
CGN	27.6±3.06[a]
DIC	22.8±2.24
SG1+CGN	24.0±4.24
SG2+CGN	26.4±3.60

SG3+CGN	25.2±4.41

Values having different Superscript, a, b,c are statistically significant ($p \leq 0.05$). (One-way ANOVA followed by Tukey's Post Hoc Test). NS: Normal Saline, CGN: Carrageenan group, DIC: 10mg/kg of Diclofenac, SG1: Group administered with 250mg/kg *Syzygium guineense,* SG2: Group administered with 500mg/kg of *Syzygium guineense,* SG3: Group administered with 1000mg/kg of *Syzygium guineense.*a= compared to Normal Saline, b= compared to 0.1% Carrageenan, c=compared to Standard drug, Diclofenac.

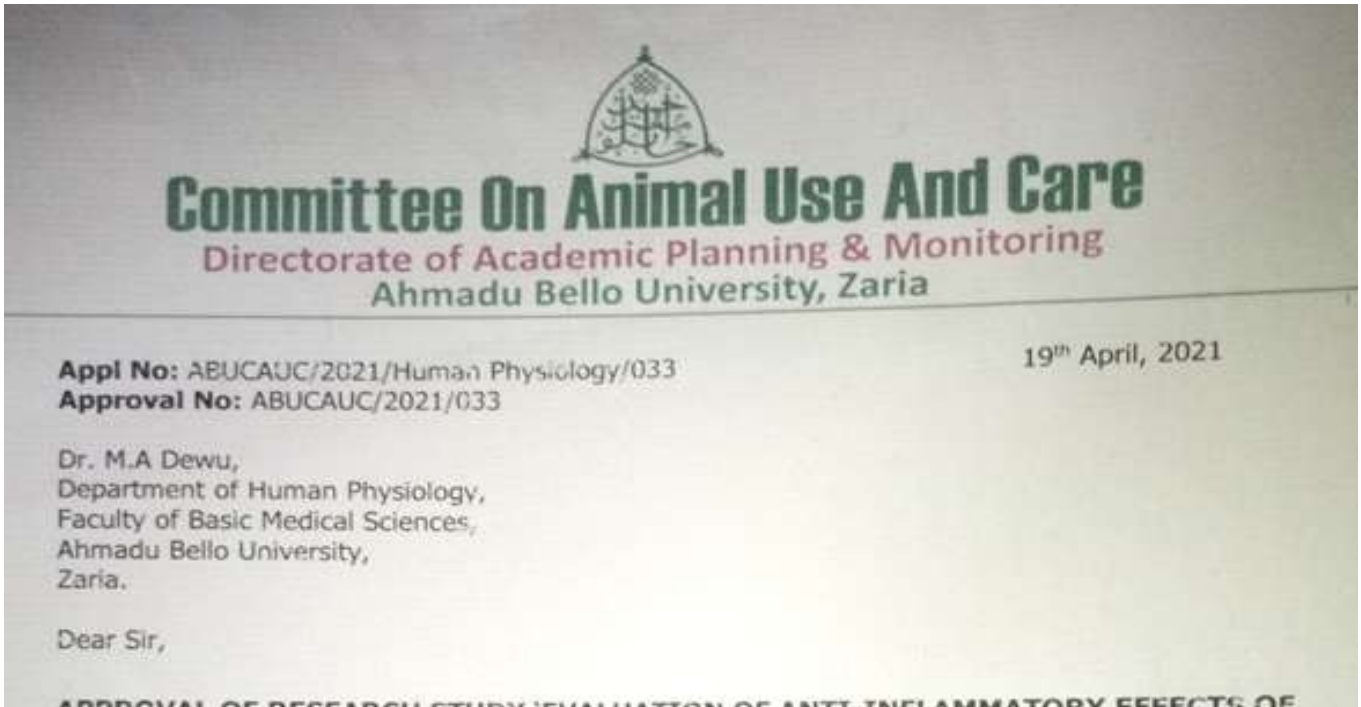
8.3Effects of *Syzygium guineense* on serum MDA, CAT, SOD, and GSH levels of male adult wistar rats.

GROUP/TREATMENT	MDA (µmol/mg	CAT (U/mg	SOD (U/ml)	GSH
NS	65.86±2.84	15.12±0.36	26.50±0.34	27.82±0.97
CGN	71.96±4.20	18.58±0.55[a]	29.04±1.15	29.76±0.47
DIC	67.84±2.4	18.54±1.23[a]	29.00±1.7	40.04±2.97[a,b]
SG1+CGN	66.28±1.56	20.44±1.00[a]	34.50±1.25[a]	32.82±1.16[c]
SG2+CGN	66.32±1.92	17.62±0.28	27.58±0.29	32.36±1.50[c]

SG3+CGN	66.38±0.24	17.94±0.57c	26.50±0.19	29.22±1.12[c]

Values having different Superscript, a, b,c are statistically significant ($p < 0.05$). (One-way ANOVA followed by Tukey's Post Hoc Test). NS: Normal Saline, CGN: Carrageenan group, DIC: 10 mg/kg of Diclofenac, SG2: Group administered with 250mg/kg *Syzygium guineense*, SG2: Group administered with 500mg/kg of *Syzygium guineense*, SG3: Group administered with 1000mg/kg of *Syzygium guineense*.a= compared to Normal Saline, b= compared to 0.1% Carrageenan, c=compared to Standard drug, Diclofenac. CAT**:** Catalase; GSH: Glutathione Concentration; SOD: Superoxide Dismutase Activity; MDA: Malondialdehyde Concetration.

8.4Ethical ApprovalLetter



Committee On Animal Use And Care

Directorate of Academic Planning & Monitoring

Ahmadu Bello University, Zaria

Appl No: ABUCAUC/2021/Human Physiology/033

Approval No: ABUCAUC/2021/033

19th April, 2021

Dr. M.A Dewu,
Department of Human Physiology,
Faculty of Basic Medical Sciences,
Ahmadu Bello University,
Zaria.

Dear Sir,

APPROVAL OF RESEARCH STUDY 'EVALUATION OF ANTI-INFLAMMATORY EFFECTS OF