TITLE PAGE

ANTI-IFLAMMATORY AND ANALGESIC EFFECTS OF METHANOL FRACTIONS OF COLA HISPIDA LEAF IN MALE ALBINO RATS

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR AWARD OF DEGREE OF MASTER OF SCIENCE (MSc) IN PHARMACOLOGICAL BIOCHEMISTRY, UNIVERSITY OF NIGERIA, NSUKKA

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CERTIFICATION

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DEDICATION

This work is dedicated to the Almighty God.

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ABSTRACT

The study is aimed at evaluating the anti-inflammatory and analgesic effects of methanol fraction of *Cola hispida* leaves on male albino rats. Cola hispida is a medicinal plant believed to contain many secondary metabolites that may be effective in the treatment of inflammation and pain. This study investigated the qualitative and quantitative phytochemical constituents, acute toxicity, *in vitro* percentage inhibition of protein denaturation, the analgesic and anti-inflammatory effects of the plant fraction. The extract did not produce mortality up to 1000 mg/kg b. w. The phytochemical analysis showed the presence of flavonoids (4.28 ± 0.28 mg/g), phenolic compounds (4.03 ± 0.03 mg/g), taninns (1.27 ± 0.04 mg/g), alkaloids (1.68 ± 0.02 mg/g), glycosides (0.84 ± 0.02 mg/g) and saponins (0.74 ± 0.06 mg/g). However terpenoid was not detected. The methanol extract of *Cola hispida* was fractionated using solvent-solvent partitioning. The three (3) solvents used for the partitioning were n hexane, ethylacetate and 20% methanol based on increasing polarity. The *in vitro* anti-inflammatory effect of *Cola hispida* was determined through the ability of the plant fractions to inhibit protein denaturation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of the plant fractions to inhibit heat induced albumin denaturation was studied. The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of the methanol fraction so that final concentrations become 100, 200, 300, 400, 500 µg/ml. The fraction that contains 20% methanol was the most effective in inhibiting heat induced albumin denaturation, maximum inhibition of 61.15% was observed at 500 µg/ml. A total of Eighty (80) male albino rats were used. The animals were randomly divided into four (4), for the main experimental models, each experimental models was further divided into five (5) groups containing four (4) animals each. The egg albumin-induced paw oedema and agar-induced leukocyte mobilization to the peritoneal cavity methods were used to evaluate the anti-inflammatory effect of the 20% methanol fraction at 100, 200, and 400 mg/kg dose levels, the plant fraction was administered orally 1 hour before injecting the philogistic agent; whereas central and peripheral analgesic effect was evaluated using formalin-induced paw licking and acetic acid induced writhing rats models, of the plant fraction at the same dose levels. Data were analyzed using one-way ANOVA followed by Duncan's post hoc test. All tested doses of the plant fraction showed significant ($p < 0.05$) analgesic effect with maximum result of 54.83% and 71.42% inhibition of acetic acid induced writhing and formalin-induced paw licking respectively. Maximum anti-inflammatory effect was recorded at 4 hours after induction, with 50.00% reduction in egg albumin-induced paw oedema. Moreover, all tested doses of the plant fraction significantly ($p < 0.05$) inhibited leukocyte mobilization to the intraperitoneal cavity. The control had the highest total leukocyte count (9626.02) and the highest percentage of neutrophils that migrated (65.18%). They all had their maximum inhibitory effect at the highest dose (400 mg/kg) and these compared well with that of indomethacin (standard drug), which implies that the effect is dose dependent. The present study indicated that the plant fraction had both analgesic and anti-inflammatory activity, which probably depended on their ability to prevent the production of some pro-inflammatory mediators and cytokines.

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LIST OF ABBREVIATIONS

5-HETE: 5-hydroxyeicosatetranoate

BAT: Basophil Activation Test

COX: Cyclo-Oxygenases

CSFs: Colony-Stimulating Factors

CT: Condensed Tannin

DLC: Differential Leukocyte Count

DMARDS: Disease-modifying Anti-Rheumatic drugs

HT: Hydrolysable Tannin

INF-γ: Interferon γ

IFN-gamma: Interferon Gamma

IL-1: Interleukin-1

LOX: Lipoxygenases

LTB_4: Leukotriene B_4

NO: Nitric Oxide

NSAIDs: Nonsteroidal Anti-inflammatory Drugs

PAF: Platelet Activating Factor

PGs: Prostaglandins

PMN: Polymorphonuclear

PT: Phlorotannins

TLC: Total Leukocyte Count

TNF-α: Tumor Necrosis factor alpha

TXA_2: Thromboxane A_2

CHAPTER ONE

INTRODUCTION

Inflammation is a natural immune system response to infection or injury, it is essential to sustain the normal tissue homeostasis. Inflammation is a body defense reaction in order to eliminate or limit the spread of injurious agent, it can be triggered by a number of factors; such as pathogens, damaged cells, toxic compounds, or irradiation (Goyal *et al.*, 2013). The classical symptoms of inflammation for many years now include swelling, redness, pain and heat (Hassan *et al.*, 2015).

According to the International Association for the Study of Pain (IASP), pain is an unpleasant, sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Wang *et al.*, 2014). The causes of inflammation can be infectious or non-infectious. Inflammation can be beneficial when, for example, your knee sustains a blow and tissues need care and protection; albeit sometimes, inflammation can persist longer than necessary, causing more harm than benefit and can eventually cause several diseases and conditions, including some cancers and rheumatoid arthritis (Arumugam *et al.*, 2012). Inflammation is the body's attempt at self-protection to remove harmful stimuli and begin the healing process. The fundamental aim of inflammatory response is to localize and eliminate the harmful agents; secondarily, to remove damaged tissue components leading to the healing of the affected body part. Infections, wounds, and any damage to tissue would not be able to heal without an inflammatory response.

An inflammatory response involves macrophages, neutrophils known to secrete different mediators that are responsible for the initiation, progression, persistence, regulation, and eventual resolution of the acute state of inflammation (Kim *et al.*, 2014). The resolution of inflammation is influenced by several anti-inflammatory mediators and the recruitment of monocytes for the removal of cell or tissue debris. It is possible that the resolution may not occur in the acute phase, thereby turning into a chronic phase.

In Nigeria the use of plants to treat many diseases is widely practiced. More than 120 plant species of 63 families are used in Nigeria to treat pain-related inflammatory disorders. The bioactive principles in these plant species have been linked to secondary metabolites such as phenolic compounds (curcumins, flavonoids and tannins), saponins, terpenoids and alkaloids (Awad *et al.*, 2013). Biological and

therapeutic properties attributed to these plant metabolites include antioxidant, anti-inflammatory, antimicrobial and anticancer activities (Sengar *et al.,* 2015). The anti-inflammatory mechanisms of action of many phenolic compounds such as flavonoids, tannins and curcumins are thought to be via their free radical scavenging activities or the inhibition of pro-inflammatory enzymes such as cyclo-oxygenases (COX) and lipoxygenases (LOX) in the inflammatory cascades (Zhang and Reddy, 2018). *Cola hispida* is known as "oji enyi" in Igbo eastern Nigeria, it is mostly found in the wild and has not being relatively explored. Cola hispida is a plant native to tropical West Africa and belongs to Sterculiaceae family and it contains bioactive constituents such as flavonoids and tannins (Neuwinger, 2000). In some parts of Nigeria and Africa it is used as medicines for the treatment of stomach troubles, cutaneous, subcutaneous parasitic infection; A leaf-decoction is taken as a tonic in Congo, to ease cough (Neuwinger, 2000). The present study was conducted to evaluate the anti-inflammatory and analgesic effect of *Cola hispida* leaf fraction on male albino rats.

1.1 Types of Inflammation

Inflammatory process has two phases: acute and chronic. The acute inflammation occurs a few minutes after tissue damage or infection (Abbas *et al.,* 2014). An inflammatory response that lasts only a few days is called acute inflammation, while a response of longer duration is referred to as chronic inflammation. Acute inflammation is characterized by an increase in permeability of blood vessels, extravasation of fluid and proteins and accumulation of white blood cells for a short period (Bhagyasr *et al.*, 2015). The primary mediators of acute inflammation include histamine, serotonin, and C0X-2 (Abdulkhaleg *et al.*, 2018). The failure of the management of acute inflammation and an autoimmune response to a self-antigen lead to chronic inflammation and disease (Kalinski, 2012). Chronic inflammation is mediated by inflammatory mediators such as Prostaglandin E2, nitric oxide and lipoxygenases (Edwards, 2019). Chronic inflammation may result in ailments such as chronic peptic ulcers, rheumatoid arthritis, systemic lupus, asthma, chronic periodontitis and cancer. Several things can cause chronic inflammation, including:

- Untreated causes of acute inflammation, such as an infection or injury
- An autoimmune disorder, which involves your immune system mistakenly attacking healthy tissue

- Long-term exposure to irritants,
- such as industrial chemicals or polluted air

In addition, some cases of chronic inflammation do not have a clear underlying cause. Experts also believe that a range of factors may also contribute to chronic inflammation, such as: Smoking, obesity, alcohol and chronic stress (Sowemimo *et al.*, 2015).

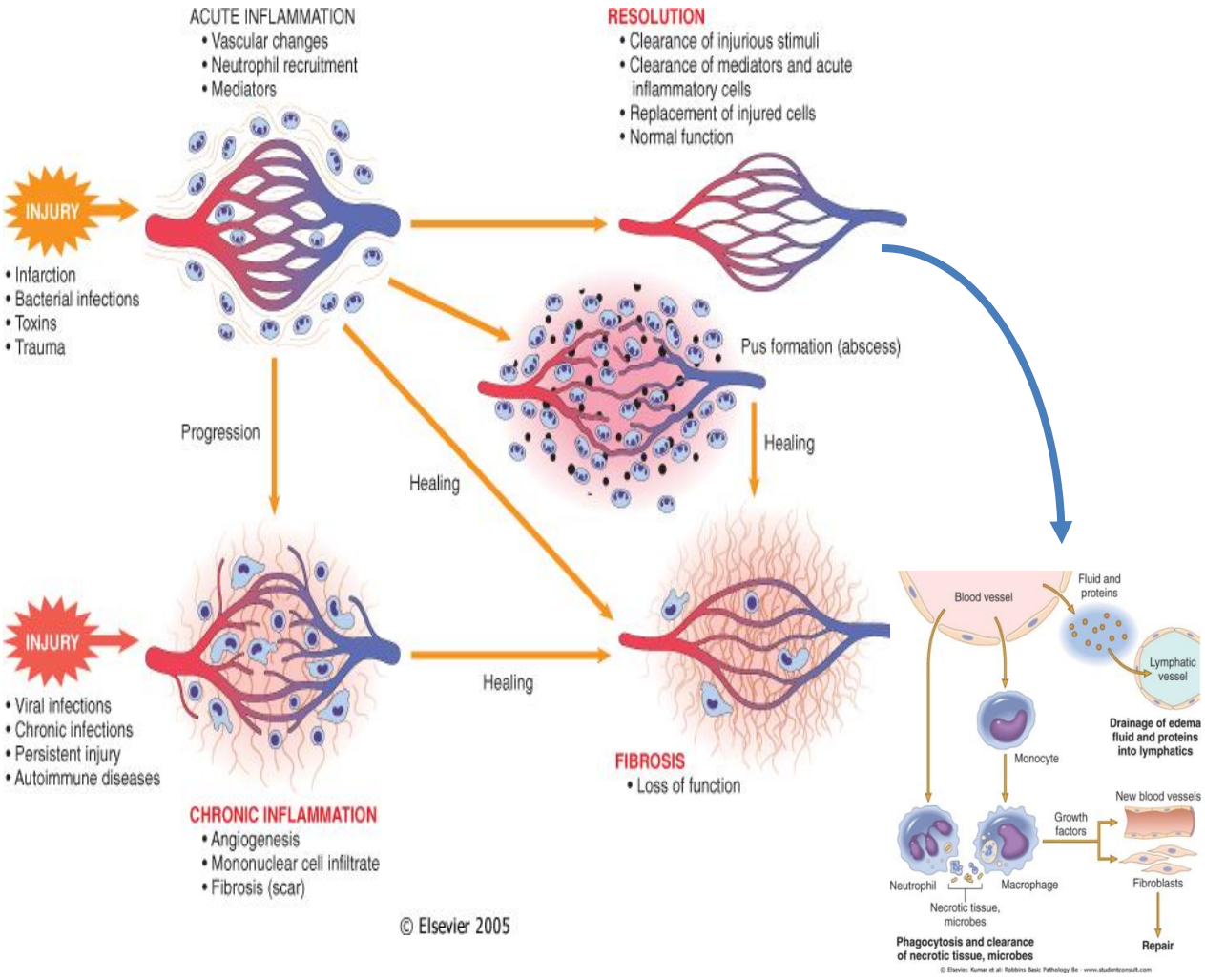

Fig 1: Types of inflammation

Source: (Bhagyasr *et al.*, 2015)

1.2 Components of Inflammation

A complex interplay of inflammatory cells and chemical mediators are responsible for inflammation (Bubious *et al.*, 2011). It is now understood that the inflammatory process consists of an early-phase response involving mast cell degranulation with the release of histamine and a late-phase, response characterized by the migration of inflammatory cells. This can be broadly divided into a vascular phase (Biochemical mediators) that occurs first, followed by a cellular phase involving immune cells (Nair *et al.*, 2012).

1.3 Vascular components

Biochemical mediators released during inflammation intensify and propagate the inflammatory response. These mediators are soluble, diffusible molecules that can act locally and systemically (Serhan, 2008). Mediators derived from plasma include complement and complement-derived peptides and kinins. Complement-derived peptides (C3a, C3b, and C5a) are released via the classic or alternative pathways of the complement cascade, they increase vascular permeability, cause smooth muscle contraction, activate leukocytes, and induce mast-cell degranulation. Complement-derived peptide C5a is a potent chemotactic factor for neutrophils and mononuclear phagocytes (Andreas *et al.*, 2013). Kinins are also important inflammatory mediators.

1.3.1 Bradykinin

The most important kinin is bradykinin, which increases vascular permeability and vasodilation and, importantly, activates phospholipase A_2 (PLA_2) to liberate arachidonic acid (AA). Bradykinin is also a major mediator involved in the pain response. Other mediators are derived from injured tissue cells or leukocytes recruited to the site of inflammation (Emert *et al.*, 2013). Mast cells, platelets, and basophils produce the vasoactive amines serotonin and histamine.

1.3.2 Histamine

Histamine causes arteriolar dilation, increased capillary permeability, contraction of nonvascular smooth muscle, and eosinophil chemotaxis and can stimulate nociceptors responsible for pain response. Its release is stimulated by the complement components C3a and C5a and by lysosomal

proteins released from neutrophils (Zhu *et al.*, 2011). Histamine activity is mediated through the activation of one of four specific histamine receptors, designated H_1, H_2, H_3, or H_4 in target cells. Most histamine-induced vascular effects are mediated by H_1 receptors. Histamine H_2 receptors mediate some vascular effects but are more important for their role in histamine-induced gastric secretion. Less is understood about the role of H_3 receptors, which may be localized to the central nervous system (Panula *et al.*, 2015). Histamine H_4 receptors are located on cells of hematopoietic origin, and H_4 antagonists are promising drug candidates to treat inflammatory conditions involving mast cells and eosinophils (allergic conditions).

1.3.3 Serotonin

Serotonin (5-hydroxytryptamine) is a vasoactive mediator similar to histamine found in mast cells and platelets in the Gastro intestinal tract and Central Nervous System. Serotonin also increases vascular permeability, dilates capillaries, and causes contraction of nonvascular smooth muscle (Sol and Fresno, 2015). In some species, including rodents and domestic ruminants, serotonin may be the predominant vasoactive amine.

1.3.4 Cytokines

Cytokines, including interleukins 1–10, tumor necrosis factor α (TNF-α), and interferon γ (INF-γ) are produced predominantly by macrophages and lymphocytes but can be synthesized by other cell types as well (Biswas and Mantovani, 2014). Their role in inflammation is complex. These polypeptides modulate the activity and function of other cells to coordinate and control the inflammatory response (Mills, 2012). Two of the more important cytokines, interleukin-1 (IL-1) and TNF-α, mobilize and activate leukocytes, enhance proliferation of B cells and T cells and natural killer cell cytotoxicity, and are involved in the biologic response to endotoxins (Isailovic *et al.*, 2015). IL-1, IL-6, and TNF-α mediate the acute phase response and pyrexia that may accompany infection and can induce systemic clinical signs, including sleep and anorexia. In the acute phase response, interleukins stimulate the liver to synthesize acute-phase proteins, including complement components, coagulation factors, protease inhibitors, and metal-binding proteins (Nelson, 2014). By increasing intracellular calcium ion concentrations in leukocytes, cytokines are also important in the induction of Phospholipase A_2. Colony-stimulating factors (GM-CSF, G-CSF,

and M-CSF) are cytokines that promote expansion of neutrophil, eosinophil, and macrophage colonies in bone marrow (Nelson, 2014). In chronic inflammation, cytokines IL-1, IL-6, and TNF-α contribute to the activation of fibroblasts and osteoblasts and also enhances the release of enzymes such as collagenase and stromelysin that can cause cartilage and bone resorption (Buer, 2014). Experimental evidence also suggests that cytokines stimulate synovial cells and chondrocytes to release pain-inducing mediators. Lipid-derived autacoids play important roles in the inflammatory response and are a major focus of research into new anti-inflammatory drugs. These compounds include the eicosanoids such as prostaglandins, prostacyclin, leukotrienes, and thromboxane A and the modified phospholipids such as platelet activating factor (PAF). Eicosanoids are synthesized from 20-carbon polyunsaturated fatty acids by many cells, including activated leukocytes, mast cells, and platelets and are therefore widely distributed. Hormones and other inflammatory mediators (TNF-α, bradykinin) stimulate eicosanoid production either by direct activation of Phospholipase A_2, or indirectly by increasing intracellular Ca^{2+} concentrations, which in turn activate the enzyme. Cell membrane damage can also cause an increase in intracellular Ca^{2+}. Activated Phospholipase A_2 hydrolyzes arachidonic acid, which is rapidly metabolized via one of two enzyme pathways—the cyclooxygenase (COX) pathway leading to the formation of prostaglandin and thromboxanes, or the 5-lipoxygenase (5-LOX) pathway that produces the leukotrienes (Bitencourt *et al.*, 2013).

Lipoxygenase is found predominately in platelets, leukocytes, and the lungs, 5-LOX catalyzes the formation of unstable hydroxyperoxides from arachidonic acid. These hydroxyperoxides are subsequently converted to peptide leukotrienes.

1.3.5 Leukotrienes

Leukotriene B_4 (LTB_4) and 5-hydroxyeicosatetranoate (5-HETE) are strong chemoattractants stimulating polymorphonuclear leukocyte movement. Leukotriene B_4 also stimulates the production of cytokines in neutrophils, monocytes, and eosinophils and enhances the expression of C3b receptors (Bitencourt *et al.*, 2013). Other leukotrienes facilitate the release of histamine and other autacoids from mast cells and stimulate bronchiolar constriction and mucous secretion. In some species, leukotrienes C_4 and D_4 are more potent than histamine in contracting bronchial smooth muscle

1.3.6 Prostaglandin

Prostaglandin D_2, prostaglandin E_2, prostaglandin F_2 and prostaglandin I_2 (which is also known as prostacyclin); are collectively called prostaglandins. They are a group of lipids produced at the site where they are needed. Prostaglandins are produced in nearly all cells and are part of the body's way of dealing with injury and illness (Newton and Roberts, 2016). Prostaglandins are made at sites of tissue damage or infection, where they cause inflammation, pain and fever as part of the healing process. When a blood vessel is injured, a prostaglandin called thromboxane stimulates the formation of a blood clot to try to heal the damage; it also causes the muscle in the blood vessel wall to contract (causing the blood vessel to narrow) to try to prevent blood loss. Another prostaglandin called prostacyclin has the opposite effect to thromboxane, reducing blood clotting and removing any clots that are no longer needed; it also causes the muscle in the blood vessel wall to relax, so that the vessel dilates. The opposing effects that thromboxane and prostacyclin have on the width of blood vessels can control the amount of blood flow and regulate response to injury and inflammation (Newton and Roberts, 2016).

The chemical reaction that makes the prostaglandins involves several steps; the first step is carried out by an enzyme called cyclooxygenase. There are two main types of this enzyme: cyclooxygenase-1 and cyclooxygenase-2. When the body is functioning normally, baseline levels of prostaglandins are produced by the action of cyclooxygenase-1. When the body is injured (or inflammation occurs in any area of the body), cyclooxygenase-2 is activated and produces extra prostaglandins, which help the body to respond to the injury (Kalinski, 2012). Cyclooxygenase catalyzes the oxygenation of Arachidonic acid to form the cyclic endoperoxide PGG_2, which is converted to the closely related PGH_2. Both PGG_2 and PGH_2 are inherently unstable and rapidly converted to various prostaglandins, thromboxane A_2 (TXA_2), and prostacyclin (PGI_1). In the vascular beds of most animals, PGE_1, PGE_2, and PGI_1 are potent arteriolar dilators and enhance the effects of other mediators by increasing small-vein permeability.

Other prostaglandins, including $PGF_{2\alpha}$ and thromboxane, cause smooth muscle contraction and vasoconstriction (Da siva *et al.*, 2014). Prostaglandins sensitize nociceptors to pain-provoking mediators such as bradykinin and histamine and, in high concentrations, can directly stimulate sensory nerve endings. Thromboxane A_2 is a potent platelet-aggregating agent involved in

thrombus formation. Prostaglandins carry out their actions by acting on specific receptors; at least eight different prostaglandin receptors have been discovered. The presence of these receptors in different organs throughout the body allows the different actions of each prostaglandin to be carried out, depending on which receptor they interact with. Prostaglandins are very short-lived and are broken down quickly by the body. They only carry out their actions in the immediate vicinity of where they are produced; this helps to regulate and limit their actions (Kalinski, 2012).

1.3.7 Platelet activating factor

Platelet activating factor (PAF) is also derived from cell membrane phospholipids by the action of phospholipase A_2. Platelet activating factor, synthesized by mast cells, platelets, neutrophils, and eosinophils, induces platelet aggregation and stimulates platelets to release vasoactive amines and synthesize thromboxanes (Bitencourt *et al.*, 2013). Platelet activating factor also increases vascular permeability and causes neutrophils to aggregate and degranulate.

1.3.8 Nitric Oxide

The role of the free radical gas nitric oxide (NO) in inflammation is well established. Nitric Oxide is an important cell-signaling messenger in a wide range of physiologic and pathophysiologic processes (Bomi *et al.*, 2017). Small amounts of NO play a role in maintaining resting vascular tone, vasodilation, and antiaggregation of platelets. In response to certain cytokines (TNF-α, IL-1) and other inflammatory mediators, the production of relatively large quantities of NO is stimulated. In larger quantities, NO is a potent vasodilator, facilitates macrophage-induced cytotoxicity, and may contribute to joint destruction in some types of arthritis (Chen *et al.*, 2018).

1.4 Cellular Component

The cellular component involves leukocytes, which normally reside in blood and must move into the inflamed tissue via extravasations to aid in inflammation (Wang *et al.*, 2014). Some act as phagocytes, ingesting bacteria, viruses and cellular debris. Others release enzymatic granules that destroy pathogenic invaders. Leukocytes also release inflammatory mediators that develop and maintain the inflammatory response. In general, acute inflammation is mediated by granulocytes, whereas chronic inflammation is mediated by mononuclear cells such as monocytes and lymphocytes.

1.4.1 Macrophages

Macrophages are a type of white blood cells that engulf and digest cellular debris, foreign substances, microbes, cancer cells, and anything else that does not have the types of proteins specific to the surface of healthy body cells on its surface; in a process called phagocytosis. They are found in essentially all tissues (Nelson, 2014) where they patrol for potential pathogens by amoeboid movement. They play a critical role in non-specific defence (innate immunity), and also help initiate specific defence mechanisms (adaptive immunity) by recruiting other immune cells such as lymphocytes. In humans, dysfunctional macrophages cause severe diseases such as chronic granulomatous disease that result in frequent infections. Beyond increasing inflammation and stimulating the immune system, macrophages also play an important anti-inflammatory role and can decrease immune reactions through the release of cytokines.

Macrophages that encourage inflammation are called MI macrophages, whereas those that decrease inflammation and encourage tissue repair are called M2 macrophages (Mills, 2012). This difference is reflected in their metabolism, M1 macrophages have the unique ability to metabolize arginine to the "killer" molecule nitric oxide, whereas M2 macrophages have the unique ability to metabolize arginine to the "repair" molecule ornithine. Human macrophages are about 21 micrometers (0.00083 in) in diameter (Biswas and Mantovani, 2014) and are produced by the differentiation of monocytes in tissues. Macrophages are essential for wound healing, they replace polymorph nuclear neutrophils as the predominant cells in the wound by two days after injury. Attracted to the wound site by growth factors released by platelets and other cells, monocytes from the bloodstream enter the area through blood vessel walls. Numbers of monocytes in the wound peak one to one and a half day after the injury occurs. Once they are in the wound site, monoctyes mature into macrophages. The spleen contains half the body's monocytes in reserve ready to be deployed to injured tissue (Abdulkhaleg *et al.*, 2018). The macrophage's main role is to phagocytize bacteria and damaged tissue, and they also debride damaged tissue by releasing proteases (Nelson, 2014). Macrophages also secrete a number of factors such as growth factors and other cytokines, especially during the third and fourth post-wounding days. These factors attract cells involved in the proliferation stage of healing to the area. Macrophages may also restrain the contraction phase (Nelson, 2014). Macrophages are stimulated by the low oxygen content of their surroundings to produce factors that induce and speed angiogenesis (Tak et al., 2013) and they also stimulate cells

that re-epithelialize the wound, create granulation tissue, and lay down a new extracelluar matrix (Mills, 2012). By secreting these factors, macrophages contribute to pushing the wound healing process into the next phase.

1.4.2 Monocytes

Monocytes are a type of white blood cells (leukocytes). They are the largest of all leukocytes. They are part of the innate immune system of vertebrates including all mammals (humans included), birds, reptiles and fish. They are amoeboid in shape, having agranulated cytoplasm. Monocytes have unilobar nuclei which makes them one of the types of mononuclear leukocytes (containing azurophil granules) (Kaur *et al.*, 2014). The archetypal idea of the nucleus is that it is bean-shaped or kidney-shaped, although the most important distinction is that it is not deeply furcated into lobes, as occurs in polymorphonuclear leukocytes. Monocytes constitute 2% to 10% of all leukocytes in the human body. They play multiple roles in immune function. Such roles include replenishing resident macrophages under normal states; and in response to inflammation signals, monocytes can move quickly (Aprox. 8-12 hours) to sites of infection in the tissues and divide/differentiate into macrophages and dendritic cells to elicit an immune response. Half of them are stored in the spleen (Umamageswan *et al.*, 2015).

Monocytes are usually identified in stained smears by their large kidney shaped or notched nucleus. These changes into macrophages after entering into the tissue spaces, and in endothelium can transform into foam cells. Monocytes are produced by the bone marrow from precursors called monoblasts, bipotent cells that differentiated from hemotopoietic stem cells. Monocytes circulate in the bloodstream for about one to three days and then typically move into tissues throughout the body. They constitute between three to eight percent of the leukocytes in the blood. Half of them are stored as a reserve in the spleen in clusters in the red pulp's Cords of Billroth (Ahmed, 2011). In the tissues, monoctes mature into different types of macrophages at different anatomical locations. Monocytes are the largest corpuscles in the blood. Monocytes which migrate from the bloodstream to other tissues will differentiate into tissue resident macrophages of dendritic cells (Biswas and Mantovani, 2014). Macrophages are responsible for protecting tissues from foreign substances, but are also suspected to be important in the formulation of important organs like the heart and brain. They are cells that possess a large smooth nucleus, a large area of cytoplasm, and many internal vesicles for processing foreign material. Monocytes and their macrophage and dendritic-cell

progeny serve three main functions in the immune system. These are phagocytosis, antigen presentation and cytokine production. Phagocytosis is the process of uptake of microbes and particles followed by digestion and destruction of this material. Monocytes can perform phagocytosis using intermediary (opsonising) proteins such as antibodies or complement that coat the pathogen, as well as by binding to the microbe directly via pattern-recognition receptors that recognize pathogens. Monocytes are also capable of killing infected host cells via antibody-dependent cell-mediated cytotoxicity (Mills, 2012). Vacuolization may be present in a cell that has recently phagocytized foreign matter. Microbial fragments that remain after such digestion can serve as antigens. The fragments can be incorporated into MHC molecules and then trafficked to the cell surface of monocytes (and macrophages and dendritic cells). This process is called antigen presentation and it lead to activation of T lymphocytes, which then mount a specific immune response against the antigen (Nair *et al.*, 2012). Other microbial products can directly activate monocytes and this leads to production of pro-inflammatory mediators, with some delay of anti-inflammatory cytokines. Typical cytokines produced by monocytes are TNF, IL-1, and IL-12 (Sowemimo *et al.*, 2015).

1.4.3 Mast cells

A mast cell is a cell that is derived from the myeloid stem cell. It contains many granules rich in histamine and heparin. They are known to be involved in wound healing, angiogenesis, defence against pathogens and blood-brain barrier function (da Silva *et al*, 2014; Polyzoidis *et al*, 2015). They are also known to play roles in allergy and anaphylaxis. Mast cells look very much like basophils but are different cells as they develop from different hematopoietic stem cells. Unlike basophils, which leave the bone marrow in the mature form, mast cells circulate in an immature form and only mature when they get to their target tissue site (Biswas and Mantovani, 2014). They are seen in tissues surrounding blood vessels and nerves and also in the skin, mucosa of the lungs, digestive tract, conjunctive, nose and mount (Zhang *et al.*, 2011). Mast cells play important roles in the inflammatory process. They release histamine which dilates post-capillary venules, activates the endothelium, increases blood vessel permeability leading to local oedema and also depolarizes the nerve endings leading to pain or itching.

1.4.4 Neutrophils

Neutrophil granulocytes (also known as neutrophils) are the most abundant (40% to 75%) type of white blood cells in most mammals and form an essential part of the innate immune system. Functionality varies in different animals (Ermert *et al.,* 2013). They are formed from stem cells in the bone marrow. They are short-lived and highly motile. Neutrophils may be subdivided into segmented neutrophils and banded neutrophils (or bands). They form part of the polymorphonuclear cell family (PMNs) together with basophils and eosinophils (Tak *et al.*, 2013). The name neutrophil derives from staining characteristics on hematoxylin and cosin (H&E) histological or cytological preparations. Whereas basophilic white blood cells stain dark blue and eosinophilic white blood cells stain bright red, neutrophils stain a neutral pink. Normally, neutrophils contain a nucleus divided into 2-5 lobes. Neutrophils are a type of phagocyte and are normally found in the bloodsream. During the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure (Jacobs et al., 2010) and some cancers (Newton and Roberts, 2016), neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation. They migrate through the blood vessels, then through interstitial tissue, following chemical signals such as Interleukin-8 (IL-8), C5a, and Leukotriene B4 in a process called chemotaxis. They are the predominant cells in pus, accounting for its whitish/yellowish appearance. Neutrophils are recruited to the site of injury within minutes following trauma, and are the hallmark of acute inflammation (Ekwueme *et al.*, 2015). However, due to some pathogens being indigestible, they can be less useful alone. Neutrophil granulocytes have an average diameter of 12-15 micrometers in peripheral blood smears. When analyzing neutrophils in suspension, neutrophils have an average diameter of 12-15 micrometers (μm) in peripheral blood smears. When analyzing neutrophils in suspension, neutrophils have an average diameter of 8.85 μm. With the eosinophil and the basophil, they form the class of polymorphonuclear cells, named for the nucleus' multilobulated shape (as compared to lymphocytes and monocytes, the other types of white cells). The nucleus has a characteristic lobed appearance, the separate lobes connected by chromatin. The nucleolus disappears as the neutrophil matures, which is something that happens in only a few other types of nucleated cells (Kim *et al.*, 2014). Neutrophils are the most abundant white blood cells in humans (approximately 1011 are produced daily); they account for approximately 50-70% of all white blood cells (leukocytes). The stated normal range for human blood counts varies between laboratories, but a neutrophil count of

2.5-7.5 x 109/L is a standard normal range. People of African and Middle Eastern descent may have lower counts, which are still normal. The average lifespan of (non-activated human) neutrophils in the circulation has been reported by different studies to be between 5 and 90 hours (Tak *et al.*, 2013) upon activation, they marginate (position themselves adjacent to the blood vessel endothelium), and undergo selectin-dependent capture followed by integrin-dependent adhesion in most cases, after which they migrate into tissues, where they survive for 1-2 days. Neurophils are much more numerous than the longer-lived moncyte/macrophage phagocytes. A pathogen (disease-causing microorganism or virus) is likely to first encounter a neutrophil. Some experts hypothesize that the short lifetime of neutrophils is an evolutionary adaptation. The short lifetime of neutrophils minimizes propagation of those pathogens that parasitize phagocytes because the more time such parasites spend outside a host cell, the more likely they will be destroyed by some component of the body's defenses. Also, because neutrophil antimicrobial products can also damage host tissues, their short life limits damage to the host during inflammation. Neutrophils undergo a process called chemotaxis, which allows them to migrate toward sites of infection or inflammation. Cell surface receptors allow neutrophils to detect chemical gradients of molecules such as interleukin -8 (IL-8), interferon gamma (IFN-gamma), C3a, C5a and Leukotriene B4, which the cells use to direct the path of their migration. Being highly motile,neutrophils quickly congregate at the site of infection, attracted by cytokines expressed by activated endothelium, mast cells, and macrophages. Neutrophils express and release cytokines, which in turn amplify inflammatory reactions by several other cell types (Urlah et al., 2014).

1.4.5 Lymphocytes

A lymphocyte is one of the subtypes of white blood cell in a vertebrate's immune system. They include natural killer cells (NK cells) which function in cell-mediated, cytotoxic innate immunity, T cells (for cell-mediated, cytotoxic adaptive immunity), and B cells (for humoral, antibody-driven adaptive immunity). They are the main type of cell found in lymph, which prompted the name lymphocyte. The three major types of lymphocyte are T cells, B cells and natural killer (NK) cells (Sengar *et al.*, 2015). Lymphocytes can be identified by their large nucleus.

1.4.6 T-cells and B-cells

T cells (thymus cells) and B cells (bone marrow-or bursa-derived cells) are the major cellular components of the adaptive immune response. T cells are involved in cell-mediated immunity, whereas B cells are primarily responsible for humeral immunity (relating to antibodies). The function of T cells and B cells is to recognize specific "non-self" antigens, during a process known as antigen presentation (Oukacha *et al.,* 2018). Once they have identified an invader, the cells generate specific responses that are tailored to maximally eliminate specific pathogens or pathogen-infected cells. B cells respond to pathogens by producing large quantities of antibodies which then neutrialize foreign objects like bacteria and viruses. In response to pathogens some T cells, called cytotoxic T cells, produce toxic granules that contain powerful enzymes which induce the death of pathogen infected cells. Following activation, B cells and T cells leave a lasting legacy of the antigens they have encountered, in the form of memory cells. Throughout the lifetime of an animal, these memory cells will "remember" each specific pathogen encountered, and are able to mount a strong and rapid response if the pathogen is detected again (Nelson, 2014).

1.4.7 Natural Killer cells

Natural killer cells are a part of the innate immune system and play a major role in defending the host from both tumours and virally infected cells. Natural killer cells distinguish infected cells and tumors from normal and uninfected cells by recognizing changes of a surface molecule called MHC (major histocompatitbilty complex) class 1. Natural killer cells are activated in response to a family of cytokines called interferons. Activated NK cells release cytotoxic (cell-killing) granules which then destroy the altered cells (Da silva *et al.,* 2014). They were named "natural killer cells" because of the initial notion that they do not require prior activation in order to kill cells which are missing MHC class I.

1.4.8 Basophils

Basophil granulocytes are the least common of the granulocytes, representing about 0.01% to 0.3% of circulating white blood cells. Standard Range is 0.0-2.0% via differential blood count. Basophils contain large cytoplasmic granules which obscure the cell nucleus under the microscope when stained. However, when unstained, the nucleus is visible and it usually has two lobes. Basophils appear in many specific kinds of inflammatory reactions, particularly those that cause allergic

symptoms. Basophils contain anticoagulant heparin, which prevents blood from clotting too quickly. They also contain the vasodilator histamine, which promotes blood flow to tissues. They can be found in unusually high numbers at sites of ectoparasite infection, e.g., ticks, like eosinophils, basophils play a role in both parasitic infections and allergies (Panula *et al.*, 2015).

They are found in tissues where allergic reactions are occurring and probably contribute to the severity of these reactions. Basophils have protein receptors on their cell surface that bind immunoglobin E antibody that confers a selective response of these cells to environmental substances, for example, pollen proteins or helminth antigens. Recent studies in mice suggest that basophils may also regulate the behavior of T cells and mediate the magnitude of the secondary immune response (Nakanishi, 2010). Basophils arise and mature in bone marrow. When activated, basophils degranulate to release histamine, proteoglycans (e.g. heparin and chondroitin), and proteolytic enzymes (e.g elastase and lysophospholipase). They also secrete lipid mediators like leukotrienes (LTD-4), and several cytokines. Histamine and proteoglycans are pre-stored in the cell's granules while the other secreted substances are newly generated. Each of these substances contributes to inflammation. Recent evidence suggests that basophils are an important source of the cytokine, interleukin-4, perhaps more important than T cells. Interleukin-4 is considered one of the critical cytokines in the development of allergies and the production of IgE antibody by the immune system. There are other substances that can activate basophils to secrete which suggests that these cells have other roles in inflammation (Mills, 2012). The degranulation of basophils can be investigated in vitro by using flow cytometry and the so-called basophil-activation-test (BAT). Especially, in the diagnosis of allergies including of drug reactions (e.g. induced by contrast medium), the BAT is of great impact (Bohm *et al.*, 2011). Basopenia (a low basophil count) is difficult to demonstrate as the normal basophil count is so low; it has been reported in association with autoimmune urticarial which is a chronic itching condition (Jacobs *et al.*, 2010).

1.4.9 Eosinophils

Eosinophil granulocytes are white blood cells and one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. Along with mast cells, they also control mechanisms associated with allergy and asthma. They are granulocytes that develop during hematopoiesis in the bone marrow before migrating into blood. These cells are eosinophilic or 'acid-loving' as shown by their affinity to coal tar dyes: Normally transparent, it is

this affinity that causes them to appear brick-red after staining with eosin, a red dye, using the Romanowsky method. The staining is concentrated in small granules within the cellular cytoplasm, which contain many chemical mediators, such as histamines and proteins such as eosinophil peroxidase, ribonuclease (RNase), deoxyribonucleases (DNase), lipase, plasminogen, and major basic protein. These mediators are released by a process called degranulation following activation of the eosinophil, and are toxic to both parasite and host tissues. In normal individuals, eosinophils make up about 1-6% of white blood cells, and are about 12-17 micrometre in size (Ghildyal *et al.*, 2010). They are found in the medulla and the junction between the cortex and medulla of the thymus, and in the lower gastrointestinal tract, ovary, uterus, spleen, and lymph nodes, but not in the lung, skin, oesophagus, or some other internal organs under normal conditions. The presence of eosinophils in these latter organs is associated with disease. Eosinophils persist in the circulation for 8-12 hours, and can survive in tissue for an additional 8-12 days in the absence of stimulation (Nondo *et al.*, 2015).

These leukocytes are critically involved in the initiation and maintenance of inflammation. These cells must be able to get to the site of injury from their usual location in the blood, therefore mechanisms exist to recruit and direct leukocytes to the appropriate place. The process of leukocyte movement from the blood to the tissues through the blood vessels is known as extravasation, and can be divided up into a number of broad steps.

1.5. Inflammation Process

The process of inflammation involves changes in blood flow, destruction of tissues, increased vascular permeability and the synthesis of pro-inflammatory mediators (Abdulkhaleg, 2018). The injured cells, lymphocytes, phagocytes, mast cells and blood proteins are the sources of inflammatory mediators. The most important inflammatory mediators include bradykinins, serotonins, histamine, tumor necrosis factor-α, interleukin-6, interleukin-1β, leukotrienes, phospholipase A2, nitric oxide (NO), lipoxygenases and cyclooxygenase 2 (COX-2).

Triggered by a range of stimuli including damaged cells, pathogens and cytokines such as interleukin-6 (IL-6), IL-1β, and tumor necrosis factor alpha (TNF-α), granulocytes (such as neutrophils) and monocytes, which can then differentiate into macrophages, are attracted to the

damaged tissues through chemotaxis, amplify inflammatory reactions and initiate phagocytosis (Okeke *et al.*, 2019).

Inflammation is a dynamic process that is elicited in response to mechanical injuries, burns, microbial infections and other noxious stimuli that may threaten the well-being of the host. This process involves changes in blood flow, increased vascular permeability, destruction of tissues via the activation and migration of leucocytes with synthesis of reactive oxygen derivatives (oxidative burst) and the synthesis of local inflammatory mediators, such as prostaglandins (PGs), leukotrienes (Shah *et al.*, 2008) and platelet-activating factors induced by phospholipase A2, cyclooxygenases (COXs) and lipoxygenases. Arachidonic acid is a key biological intermediate that is converted in to a large number of eicosanoids with potent biological activities.

The two major pathways of arachidonic acid metabolism are the COX pathway, which results in the formation of both PGs and thromboxanes and the 5-lipoxygenase pathway, which is responsible for the formation of leukotrienes and 5S-hydroxy-6E, 8Z, 11Z, 14Z-eicosatetraenoic acid (5-HETE) (Ior, 2012).

During the inflammatory response, the PGE2 are at low levels in tissues with no inflammation and increase immediately in acute inflammation. As immune cells infiltrate the tissues, further increase in PGE2 levels is observed (Ahmed, 2011).

1.5.1 Resolution of inflammation

The inflammatory response must be actively terminated when no longer needed to prevent unnecessary "bystander" damage to tissues (Abbas *et al.*, 2014). Failure to do so results in chronic inflammation, and cellular destruction. Resolution of inflammation occurs by different mechanisms in different tissues (Abbas *et al.*, 2014).

Acute inflammation normally resolves by mechanisms that have remained somewhat elusive. Emerging evidence now suggests that an active, coordinated program of resolution initiates in the first few hours after an inflammatory response begins. After entering tissues, granulocytes promote the switch of arachidonic acid–derived prostaglandins and leukotrienes to lipoxins, which initiate the termination sequence (Kim *et al.*, 2014). Neutrophil recruitment thus ceases and programmed death by apoptosis is engaged. These events coincide with the biosynthesis, from omega-3 polyunsaturated fatty acids, resolvins and protectins, which critically shorten the period of

neutrophil infiltration by initiating apoptosis. As a consequence, apoptotic neutrophils undergo phagocytosis by macrophages, leading to neutrophil clearance and release of anti-inflammatory and reparative cytokines such as transforming growth factor-β1. The anti-inflammatory program ends with the departure of macrophages through the lymphatics.

1.6 Anti-inflammatory Drugs

1.6.1 Non-Steroidal Anti-inflammatory Drugs (NSAIDS): The non-steroidal anti-inflammatory drugs (NSAIDs) such as naproxen, indomethacin, ibuprofen, diclofenac, and ketoprofen are the most commonly used conventional medicinal products in the treatment of inflammation (Eddy, 2012). The NSAIDs inhibit the expression of cyclooxygenase 2 (COX-2) enzyme responsible for the production of PGE2 which induces pyrexia (Buer, 2014). However, the prolonged use of NSAIDs is linked with severe effects on the gastrointestinal tract, kidney, and cardiovascular system.

1.6.2 Steroids: Corticosteroids are a type of steroid hormone. They decrease inflammation and suppress the immune system, which is helpful when it starts attacking healthy tissue. But long-term use of corticosteroids can lead to vision problems, high blood pressure, and osteoporosis. Examples of steroidal drugs include: prednisone, cortisone and methylprednisolone. The adverse effects of steroidal drugs include weight gain, increased blood pressure, increased blood sugar, increased risk of cataracts, and avascular necrosis of bones. Steroid medications are also associated with accelerated osteoporosis (Polyzoidis, 2015).

1.6.3 Immunosuppressants: some immunosuppressive drugs are also used in the treatment of inflammatory diseases that are associated with auto-immune disease (Bomi *et al.*, 2017). Examples include Azathioprine and Disease-modifying antirheumatic drugs (DMARDS).

1.7.0 Plants as Anti-inflammatory Drugs

Traditional medicine, although still an unpopular science, is well established in some countries and traditions and has become a norm in almost 70% of population in rural areas (Sakat *et al.*, 2010). Presently synthetic drugs are dominating the market but element of toxicity that these drugs entail, cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic

administration, the most common being gastrointestinal bleeding and peptic ulcers (Yeboah and Osafo, 2017). Consequently there is a need to develop a new anti-inflammatory agent with minimum side effects. Search for a safe and effective anti-inflammatory agents have been given priority in scientific research in herbal system of medicine.

In Nigeria the use of plants to treat many diseases is widely practiced. More than 120 plant species of 63 families are used in Nigeria to treat pain-related inflammatory disorders in humans and animals. The bioactive principles in these plant species have been linked to secondary metabolites such as phenolic compounds (curcumins, flavonoids and tannins), saponins, terpenoids and alkaloids (Moghadamtousi *et al*., 2015). Biological and therapeutic properties attributed to these plant metabolites include antioxidant, anti-inflammatory, antimicrobial and anticancer activities. Common examples of herbs traditionally used to treat inflammation in Nigeria are *Alafia barteri, Combretum mucronatum*, and *Capparis thoningii Schum*. The mechanisms of action of many phenolic compounds such as flavonoids, tannins and curcumins are thought to be via their free radical scavenging activities or the inhibition of pro-inflammatory enzymes such as cyclo-oxygenases (COX) and lipoxygenases (LOX) in the inflammatory cascades.

Flavonoids are a group of polyphenols thought to inhibit the biosynthesis of prostaglandins, end-products in the COX and LOX pathways of immunologic responses. There are three known isomeric-forms of COX i.e. COX-1 and COX-2, with a recently described third isomeric-form, COX-3 that is selectively inhibited by acetaminophen and related compounds. The selective inhibition of COX-2 is more desirable because the inhibition of COX-1 in the gastric mucosa is associated with the undesirable effects of NSAIDs (Bhadrapura and Sudharshan, 2016). COX-2 is induced as an early response to pro-inflammatory mediators and stimuli such as endotoxins and cytokines. Upon induction, COX-2 synthesizes prostaglandins that contribute to inflammation, swelling and pain. Consequently, dual COX-2/LOX inhibitor compounds could potentially be developed into safer and more effective drugs for the treatment of inflammation since they could potentially inhibit biosynthesis of prostaglandins and leukotrienes respectively from arachidonic acid , without the undesirable effects of synthetic drugs.

Classic examples of herbs traditionally used to treat inflammation in Western medicine are *Matricaria chamomilla* L. and *Arnica montana* L. (Asteraceae), *Salix alba* (Salicaceae) and *Glycyrrhiza glabra* (Fabaceae). Other well-known plant products with anti-inflammatory

activity are the distillate of *Hamamelis virginiana* (witch hazel; Hamamelidaceae), *Echinacea* species including *Echinacea angustifolia* (purple coneflower; Asteraceae), *Ananas comosus* (pineapple Bromeliaceae), *Abelmoschus esculantus* (bhindi, Malvaceae) (Shah and Seth, 2010). Common examples of Asian anti-inflammatory plants are *Curcuma domestica* Val. and *Curcuma longa* L. (turmeric), *Curcuma xanthorrhiza* Roxb. (temoe-lawaq), *Zingiber officinale* Rosc. (Zingiberaceae), *Colocassia esculenta* and *Momordica charantia* (Omar *et al*., 2016).

1.7.1 Inhibitors of Lipoxygenases

Lipoxygenases are present in leukocytes, tracheal cells, keratinocytes and airway and stomach epithelium and they catalyze the introduction of a molecule of oxygen to the 5-position of arachidonic acid to give the intermediate (5S)-hydroxy-(6E, 8Z, 11Z, 14Z)-eicosatetraenoic acid or 5-HETE, which is immediately followed by the rearrangement of 5-HETE to leukotrienes. Another potential site of action for anti-inflammatory drugs is, therefore, at the level of lipoxygenases, thus inhibiting the biogenesis of leukotriene and 5-HETE. The search for specific inhibitors of lipoxygenase activity from medicinal plants results in the characterization of anti-inflammatory agents. Lipoxygenase inhibitors might hold some potential for the treatment of asthma, psoriasis, arthritis, allergic rhinitis, cancer, osteoporosis and atherosclerosis. The evidence currently available suggests the families Myrsinaceae, Clusiaceae and Asteraceae have potential as sources of lipoxygenase inhibitors.

1.7.2 Inhibitors of Phospholipase A2

Phospholipase A2 or phosphatide acylhydrolase 2, is an enzyme that catalyzes the hydrolysis of the acyl group attached to the 2-position of intracellular membrane phosphoglycerides. This hydrolysis release arachidonic acid from membrane phosphoglycerides. Arachidonic acid is the precursor of PGS, thromboxanes and leukotrienes. In regard to the possible mechanisms observed so far, the inhibition of phospholipase A2 is mediated via lipocortine or by direct interaction with the enzyme itself. The former mechanism utilizes a protein known as lipocortine, the synthesis of which is commanded by steroidal hormones and steroid like plants known as triterpenoids. Examples of lipocortine-mediated phospholipase A2 inhibitors that are of therapeutic value and potent anti-inflammatory drugs are cortisone, prednisolone and betamethasone. The other possible mechanism

involves a direct binding with the enzyme itself, a mechanism thus far unused in therapeutics, but with promise. One such compound is also a triterpene: betulinic acid (Omar *et al.*, 2016). When looking for an inhibitor of phospholipase A2 from medicinal plants, one could look into plant species that are traditionally used as snake-bite antidotes because hemolytic and myolytic phospholipases A2 are often present in snake venom, which results in damage to cell membranes, endothelium, skeletal muscle, nerves and erythrocytes.

Other medicinal features to consider when searching for plants with potential as phospholipases A2 inhibitors are plants that have abortifacient, analgesic, antipyretic and hypoglycemic uses. Such features are present in the following plant species: *Moringa oleifera, Mentha piperita and Mimosa pidoca L.*

1.7.3 Inhibitors of Cyclo-Oxygenases (COX)

An example of a medicinal plant used for the treatment of inflammation based on its activity on COX is *Harpagophytum procumbens* DC (Pedaliaceae) or devil's claw, which has long been used in South Africa for the management of pain and inflammation. Two isoforms of COX, designated COX-1 and COX-2, are known to catalyze the synthesis of PGs from arachidonic acid. A body of evidence suggests that PGs are involved in various physiopathological processes including carcinogenesis. COX-1 is present in most tissues, whereas COX-2 is inducible by carcinogens, cytokines and tumor promoters and therefore involved not only in inflammation, but also the growth of cells (Faith *et al.*, 2018). Thus, compounds that inhibit the activity of COX-2 might also be an important target for cancer chemoprevention. Nonsteroidal anti-inflammatory drugs are widely used in the treatment of pain and inflammation associated with acute injury or chronic diseases, such as rheumatoid arthritis or osteoarthritis. Classic examples of COX inhibitors of therapeutic value are aspirin, paracetamol, ibuprofen and recently introduced and withdrawn coxibs such as celecoxib. Coxibs abrogate the formation of cardioprotective PGI2, leading to a rise in blood pressure, atherogenesis and heart attack by the rupture of an atherosclerotic plaque.

There is, therefore, a need for original coxibs and one might think to look into the medicinal flora of Asia and the Pacific, as an increasing body of evidence suggests the families Apocynaceae, Clusiaceae, Asteraceae, Polygonaceae, Lamiaceae and Convolvulaceae to elaborate plant sources of biomolecules which are able to inhibit the enzymatic activity of COX (Kim *et al.*, 2014).

1.7.4 *Cola hispida*

Cola hispida is known as “oji enyi” in Igbo (Nsukka) eastern Nigeria, it is mostly found in the wild and has not being relatively explored. Cola hispida is a plant native to tropical West Africa and belongs to Sterculiaceae family and is a rich source of protein, carbohydrate, mineral salts, and bioactive constituents such as flavonoids, polyphenols and tannins (Joseph *et al.*, 2017). In some parts of Nigeria and Africa it is used as medicines for the treatment of stomach troubles, cutaneous, subcutaneous parasitic infection; A leaf-decoction is taken as a tonic in Congo, to ease cough. However, despite the importance of the *cola hispida* plant, there is a shortage of literature on this plant specie, compared to its close relative *cola acuminata* and *cola nitid*a in the genus cola (Joseph *et al*., 2017). Scientifically speaking much work has not been done on this plant.

Description: A small tree about 12 meters high, or a shrub, of the evergreen forest in the middle belt and southern Nigeria. It is medium-sized with low branches, grey bark with dark green leaves. The leaves have stalks and are alternate, oblong, leathery and tough, the leaves are up to 18 inches long and 13 inches wide. Most cola trees prefer moist, sandy, loam or clay soils that are well drained with neutral acidity (Faith *et al.*, 2018). It requires sun exposure and can tolerate drought.

Fig 2: *Cola hispida* seed and leaves

Source: (Joseph *et al*., 2017)

1.8.0 Phytochemicals

Phytochemical is a term that refers to a variety of plant-derived compounds with therapeutic activities such as anticarcinogenic, antimutagenic, antiinflammatory, and antioxidant properties. Some phytochemicals known as secondary metabolites are non-nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect humans against diseases (Mathela *et al.*, 2008). There are more than thousand known phytochemicals. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavanoids in fruits. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines, and pyrimidines of nucleic acids and chlorophyll (Omar *et al.*, 2016). Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids, and glucosides.

1.8.1 Phenols

Phenolic compounds are a main class of secondary metabolites in plants and are divided into phenolic acids and polyphenols. These compounds are found combined with mono- and polysaccharides, linked to one or more phenolic group, or can occur as derivatives, such as ester or methyl esters (Verma *et al.*, 2014). Among the several classes of phenolic compounds, the phenolic acids, flavonoids, and tannins are regarded as the main dietary phenolic compounds (Joseph *et al.*, 2017). Phenolic compounds posseses antioxidant properties that plays important role in the reduction of lipid oxidation in (plant and animal) tissues, it also reduces the risk of developing some diseases. Studies have shown that a diet rich in fruits and vegetables(source of phenolic compounds) contributes to the delay of the aging process and to the decrease of the inflammation and oxidative stress risk, related with chronic diseases (e.g cardiovascular diseases, arteriosclerosis, cancer, diabetes, cataract, disorders of the cognitive function and neurological diseases).

1.8.2 Flavonoids

Flavonoids are a group of naturally occurring polyphenolic compounds characterized by the flavan nucleus and represent one of the most prevalent classes of compounds in fruits, vegetables and plant-derived beverages. More than 8000 compounds with flavonoids structure have been identified, many of which are responsible for the attractive colors of flowers, fruits and leaves (Yeboah and Osafo, 2017). In plants, these compounds afford protection against ultraviolet radiation, pathogens, and herbivores (Kim *et al.*, 2014).

Flavonoids are considered as health promoting and disease preventing dietary supplements, like other phytonutrients, flavonoids are powerful antioxidants with anti-inflammatory and immune system benefits. Diets rich in flavonoid-containing foods are sometimes associated with cancer, neurodegenerative and cardiovascular disease prevention. Flavonoids are part of the polyphenol class of phytonutrients. Polyphenols are historically associated with skin protection, brain function, blood sugar and blood pressure regulation, in addition to antioxidant and anti-inflammatory activity (Verma *et al.*, 2014). There are several significant groups of flavonoids, including anthocyanidins, flavanols, flavones, flavonols, flavonones and isoflavones

1.8.3 Alkaloids

Alkaloids are secondary metabolites made by plants and are nitrogen containing compounds. Many alkaloids possess potent pharmacologic effects (Goyal, 2013). Drugs that contains alkaloids include cocaine, nicotine, strychnine, caffeine, morphine, pilocarpine, atropine, methamphetamine, mescaline, ephedrine, and tryptamine. Alkaloid may be classified according to the structural relationship between the nitrogen-containing structure such as pyrrolidine, piperidine, quinoline, isolquinoline and indole and the alkaloid skeleton (Heese, 2002). Generally, amino acids such as ornithine, lysine, phenylalanine, tyrosine, tryptophan and histidine are precursor for most of the alkaloids.

1.8.4 Saponins

Saponins are a diverse group of compounds widely distributed in the plant kingdom, which are characterized by their structure containing a triterpene or steroid aglycone and one or more sugar chains**.** The physiochemical and biological properties of saponins have led to a number of

traditional and industrial applications. They have traditionally been used as natural detergents. The combination of a hydrophobic aglycone backbone and hydrophilic sugar molecules confers foaming and emulsifying properties of saponins (Lorent *et al*., 2014). The name 'saponin' is derived from the Latin word 'sapo,' meaning soap, as a soapy lather forms when plants containing saponins are agitated in water. They also exhibit a variety of biological activities. Plant-derived triterpenoid and steroidal saponins have been used in the production of steroid hormones in the pharmaceutical industry, as food additives, fire extinguishers and in other industrial applications. Other interesting biological applications include their use in anti-inflammatory, hypocholesterolemic and immune-stimulating remedies (Lorent *et al.*,2014).

1.8.5 Tannins

Tannins are the most abundant secondary metabolites in plants and are well known as one of the major groups of antioxidants polyphenols. They are abundantly distributed in leaves, wood, tree bark, fruit, and roots. Indeed, tannin accounts for 5–10% of the dry weight of plant leaves (Barbehenn *et al.*, 2011). Various bioactive molecules with therapeutic effects on human diseases have been isolated from many traditional plants including medicinal plants, aromatic plants, vegetables, and fruits (Zhang and Reddy, 2018). Among these, tannins are some of the many phytochemicals and have various pharmacological activities against many chronic diseases such as cardiovascular disease, inflammatory diseases, cancer, obesity and diabetes due to their high antioxidant activity (Kumari and Jain, 2012).Tannins are found mainly in the bark, stems, seeds, roots, buds, and leaves (Barbehenn *et al.*, 2011). Tannins are either galloyl esters, or they are oligomeric and polymeric proanthocyanidins. They protect trees from fungi, pathogens, insects, and herbivorous animals (Khanbabaee and van, 2001). Tannins have been classified into three major groups: hydrolysable tannin (HT), phlorotannins (PT), and condensed tannin (CT). Hydrolysable tannins are compounds with polyol (d-glucose) esterified by phenolic groups and include gallic acid and ellagic acid (Hemingway and Karchesy, 2014). Condensable tannins are oligomers or polymers of polyhydroxy flavan-3-ol unit (polyphenolic bioflavonoids) and include catechin and epicatechin. Hydrolysable tannins are usually distributed in low amounts in plants, while CTs are abundantly or widely distributed in plants (Koleckar *et al.*, 2008).

1.8.6 Glycosides

Glycosides are compounds containing a carbohydrate and a noncarbohydrate residue in the same molecule.The carbohydrate residue is attached by an acetal linkage at carbon atom 1 to a noncarbohydrate residue or Aglycone. The nonsugar component is known as the Aglycone. The sugar component is called the Glycone (Mathela *et al.*, 2008). Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis,(Omar, 2016) which causes the sugar part to be broken off, making the chemical available for use. For this reason, most glycosides can be classified as prodrugs as they remain inactive until they are hydrolyzed, leading to the release of aglycogen, the active constituent. The classification of glycosides is based on the nature of the aglycogen, which can be any of a wide range of molecular types including phenols, quinines, terpenes, and steroids. These glycosides are heterogeneous in structure and are not easy to study as a specific group. Glycosides are of great significance as they link monosaccharides together to form oligosaccharides and polysaccharides (Sun *et al*., 2015). A classs of glycosides called steroidal glycosides are used in the treatment of heart diseases, e.g., congestive heart failure historically**;** Steroidal glycosides or cardiac glycosides (Omar, 2016). Here the aglycone part is a steroidal nucleus that is found in the plant genera *Digitalis*, *Scilla*, and *Strophanthus*. Other agents are preffered now because they don't improve survivability.

1.8.7 Terpenes

Terpenes belong to the biggest class of secondary metabolites and basically consist of five carbon isoprene units which are assembled to each other (many isoprene units) by thousands of ways. Terpenes are simple hydrocarbons, while terpenoids are modified class of terpenes with different functional groups and oxidized methyl group moved or removed at various positions (Augustin *et al*., 2011). Terpenoids are divided into monoterpenes, sesquiterpenes, diterpenes, sesterpenes, and triterpenes depending on its carbon units. Most of the terpenoids with the variation in their structures are biologically active and are used worldwide for the treatment of many diseases. Many terpenoids inhibited different human cancer cells and are used as anticancer drugs such as Taxol and its derivatives (Chen et al., 2018). Many flavorings and nice fragrances are consisting on terpenes because of its nice aroma. Terpenes and its derivatives are used as antimalarial drugs such as artemisinin and related compounds. Meanwhile, terpenoids play a diverse role in the field of

foods, drugs, cosmetics, hormones, vitamins, and so on. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers (Omar *et al.*, 2016). A research carried out by a team of biochemist showed that a tarpenoid compound -OH-isoegomaketone [(2*E*)-1-(3-furanyl)-4-OH-4-Me-2-penten-1-one isolated from the leaves of *Perilla frutescens var. crispa* exhibited inhibitory activity on nitric oxide (NO) production in lipopolysaccharide which is an inflammatory mediator(Bomi et al., 2017).The antitumor activity of essential oils of many species has been related to the presence of monoterpenes in their composition(Marianna *et al.*, 2014) .

Justification of the Study

Non-steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs and immunosuppressants used for the relief of inflammatory diseases, require long-term treatment and their use is often associated with serious side effects. This has led to the search for alternative treatments that are cheaper and have lesser side effects. Medicinal plants contain secondary metabolites that are very effective in the treatment of inflammation and pain. *Cola hispida* is a medicinal plant that have been shown to contain majority of this secondary metabolites that maybe effective in the treatment of inflammation and pain. `

1.9.0 Aim and Specific Objectives of the Study

1.9.1 Aim of the Study

The aim of this research was to evaluate the *in vitro* and *in vivo* anti-inflammatory and analgesic effects of *Cola hispida* leaf fractions.

1.9.2 Specific Objectives of the study

1. To determine the qualitative and quantitative phytochemical constituents of *Cola hispida* leaves extract.
2. To carry out acute toxicity study of the leaf extract.
3. To Fractionate the *Cola hispida* methanol extract using n hexane, ethylacetate and 20% methanol through solvent-solvent partitioning.
4. To carry out an *in vitro* anti-inflammatory evaluation of the different fractions.

5. To determine the effect of 20% methanol fraction of *Cola hispida* on egg albumin induced paw oedema in male albino rats.
6. To determine the effect of 20% methanol fraction of *Cola hispida* on formalin induced pain in male albino rats.
7. To determine the analgesic effect of 20% methanol fraction *Cola hispida* on acetic acid induced writhing in male albino rats.
8. To determine the effect of 20% methanol fraction of *Cola hispida* on agar induced leucocyte mobilization to the peritoneal cavity.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Instruments and Equipment

The equipment used was obtained from the Department of Biochemistry, University of Nigeria Nsukka and other scientific shops in Onitsha main market, Anambra State. They included: Conical flasks (Pyrex, England), Water Bath (Gallenkamp, England), Beakers (Pyrex, England), Weighing balance (Metler HAS, U.S.A), Filter papers (Whatman), Test tubes (Pyrex, England), Measuring cylinder (Pyrex, England), Glass funnel (Pyrex, England), Spectrophotometer (Spectronic 20D, Germany), Water bath (Gallenkamp, England), Refrigerator (Thermocool, England), centrifuge (Vickas Ltd, England), vernier calipper and Rotary evaporator.

2.1.2 Chemicals and Reagents

Sterile distilled water was used in the preparation of some chemicals, reagents and drugs.

Normal saline: Normal saline solution was prepared by dissolving 0.9g of NaCl in 100 ml of distilled water.

Phosphate buffered saline: This solution was prepared by dissolving 3.90g of $NaH_2PO_4.2H_2O$ was mixed with 9.5ml of $NaH_2PO_4.2H_2O$ solution and made up to 100ml with distilled water. Then 0.9g of Nacl was added to the buffer to form phosphate buffered saline.

20% Methonl: This was prepared by dissolving 20ml of 95% methanol in 80ml of water.

2.2 Methods

2.2.1 Preparation of Plant Material

The leave sample was washed with distilled water, air-dried at room temperature and pulverized into powder for extraction. The powder (1000 g) was macerated in 1.5L of methanol and allowed to stand for 48 hours at room temperature. The mixture was filtered with Whatman No. 1 filter paper and the filtrate was concentrated using rotary evaporator to get a chocolate-like semi solid extract.

The percentage yield was calculated using the formula,

$$\text{Percentage yield} = \frac{\text{Weight of Extract} \times 100}{\text{Weight of Pulverized Plant sample}}$$

2.2.2 Solvent-Solvent Partitioning

The methanol extract of *Cola hispida* was fractionated using solvent-solvent portioning. The three (3) solvents used for the partitioning were n hexane, ethylacetate and 20% methanol based on increasing polarity. The plant extract (20 g) was dissolved in 100 ml of 20% methanol and poured into a separating funnel. Then, n hexane (250 ml) was added to the mixture and shaken vigorously. The mixture was allowed to settle giving rise to two layers. The different 20% methanol and n hexane fractions were collected separately. The process was repeated until no observable colour change in the 20% methanol fraction was detected. Then, ethylacetate was used to repeat the process.

2.2.3 Qualitative Phytochemical Screening

The screening for some chemical constituents of the plant stem bark was carried out as described by Harbone (1973), and Trease and Evans (2002). Quantitative analysis was carried out as described by Harbone (1998) and Soni and Sosa (2013).

2.2.3.1 Test for Alkaloids

A quantity of the sample (0.2 g) was boiled with 5 ml of 2 % HCl on a steam bath. The mixture was filtered and 1 ml of the filtrate was treated with 2 drops of the following reagents:

(i) Dragendorff's reagent: An orange precipitate indicated the presence of alkaloids.

(ii) Mayer's reagent: A creamy-white precipitate indicated the presence of alkaloids.

(iii) Wagner's reagent: A reddish-brown precipitate indicated the presence of alkaloids.

(iv) Picric acid (1 %): A yellow precipitate indicated the presence of alkaloids.

2.2.3.2 Test for Flavonoids

A quantity of the sample (0.2 g) was heated with 10 ml ethyl acetate in boiling water for 3 minutes. The mixture was filtered, and the filtrate was used for the following tests:

(i) Ammonium test: 4 ml of the filtrate was shaken with 1 ml of dilute ammonium solution to obtain two layers. The layers were allowed to separate. A yellow precipitate observed in the ammonium layer indicated the presence of flavonoids.

(ii) Aluminum chloride test: 4 ml of the filtrate was shaken with 1 ml of 1 % aluminium chloride solution and observed for light yellow colouration that indicated the presence of flavonoids.

2.2.3.3 Ferric chloride Test for Phenolic Compounds

About 2.0 ml of the extract was measured in a test tube and 0.01 $moldm^{-3}$ Ferric chloride solution was added drop by drop. Appearance of bluish black precipitate indicated the presence of phenolic compounds and tannins.

2.2.3.4 Test for Glycosides

A quantity of the sample (2.0 g) was mixed with 30 ml of distilled water and 15 ml of dilute sulphuric acid respectively and heated in a water bath for 5minutes. The mixtures were filtered and the filtrates used for the following test.

(i) To 5ml of each of the filtrate, 0.3 ml of Fehling's solutions A and B was added until it turned alkaline (tested with litmus paper) and heated on a water bath for 2 minutes. A brick-red precipitate indicated the presence of glycosides.

2.2.3.5 Test for Saponins

A quantity of the sample (0.1 g) was boiled with 5ml of distilled water for 5 minutes. The mixture was filtered while still hot. The filtrate was used for the following tests.

(i) Emulsion test: A quantity of the filtrate (1 ml) was added to two drops of olive oil. The mixture was shaken and observed for the formation of emulsion.

(ii) Frothing test: A quantity, 1 ml of the filtrate was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for a stable froth.

2.2.3.6 Test for Tannins

A quantity of the sample (2 g) was boiled with 5ml of 45 % ethanol for 5 minutes. The mixture was cooled and then filtered and the filtrate was treated with the following solutions.

(i) Lead sub acetate solution: To 1 ml of the filtrate, 3 drops of lead sub acetate solution was added. A gelatinous precipitate indicated the presence of tannins.

(ii) Bromine water: To 1 ml of the filtrate was added 0.5 ml of bromine water and then observed for a pale brown precipitate.

(iii) Ferric chloride solution: a quantity, 1ml of the filtrate was diluted with distilled water and then 2 drops of ferric chloride solution was added. A transient greenish to black colour indicated the presence of tannins.

2.2.3.7 Test for Terpenoids and Steroids

Ethanol (9 ml) was added to 1 g of the sample and refluxed for 10 minutes and filtered. The filtrate was concentrated to 2.5 ml on a boiling water bath, and 5 ml of hot water was added. The mixture was allowed to stand for 1hour, and the waxy matter filtered off. The filtrate was extracted with chloroform (2.5 ml) using a separating funnel. To 0.5 ml of the chloroform extract in a test tube was carefully added 1ml of concentrated sulphuric acid to form a lower layer. A reddish-brown interface showed the presence of steroids.

Another 0.5 ml aliquot of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 minutes on water. A grey colour indicated the presence of terpenoids.

2.2.4 Quantitative Phytochemical Analyses of *Cola hispida* leaf Extract

2.2.4.1 Concentration of Alkaloids

The method described by Harbone (1998) was used to determine the amount of alkaloids present in the *Cola hispida* extract. A quantity of 5 g of the dried powder of each sample was weighed into a

250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath until it reached one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed.

2.2.4.2 Concentration of Saponins

The method described by Soni and Sosa (2013) was used for saponin determination. A quantity of 20 g of the *Cola hispida* extract was placed into a conical flask and 100 ml of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and saponin content was calculated.

2.2.4.3 Concentration of Flavonoids

The method described by Soni and Sosa (2013) was used for flavonoid determination. 10 g of the plant sample was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 4. The filtrate was allowed to evaporate to dryness over a water bath and weighed.

2.2.4.4 Concentration of Terpenoids

The method reported by Ladan *et al.* (2014) was used for the quantitative determination of terpenoids. Extract (2g) was weighed and soaked in 50 ml of 95 % ethanol for 24 hrs. The mixture was filtered and the filtrate extracted with petroleum ether and concentrated to dryness. The dried ether extract was treated as total terpenoids.

2.2.5 Acute Toxicity Test of Methanol Extract of *Cola hispida*

The acute toxicity test of the *Cola hispida* extract was carried out according to the method of Lorke (1983). Eighteen (18) albino mice were used in this study. The test involved two phases. In phase one, the animals were divided into three (3) groups of three (3) mice each and were given 10, 100 and 1000 mg/kg body weight of the extracts respectively and in the second phase, 1600, 2900 and 5000mg/kg body weight of the extracts were administered to the animals. The route of administration of the extract was oral.

2.2.6 Treatment

A total of Eighty (80) male albino rats were used. The animals were randomly divided into four (4), for the main experimental models, each experimental models was further divided into five (5) groups containing four (4) animals each. After one week of acclimatization, methanol fraction of Cola hispida and the standard drug (indomethacin) was administered as follows:

Groups	**Treatments**
Group 1	Normal saline
Group 2	5 mg/kg b.wt Indomethacin
Group 3	100 mg/kg b.wt 20% methanol fraction
Group 4	200 mg/kg b.wt 20% methanol fraction
Group 5	400 mg/kg b.wt 20% methanol fraction

2.2.7 Evaluation of the *In VItro* Anti-inflammatory effect

The *in vitro* anti-inflammatory effect of *Cola hispida* was determined using the method described by Urlah *et al.* (2014). The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of the methanol fraction so that final concentrations become 100, 200, 300, 400, 500 µg/mL.

Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37°C ± 2) in a BOD incubator (Lab line Technologies) for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle (normal saline) as blank. Acetyl salicylic acid at the final concentration of (100, 200, 300, 400, 500µg/mL) was used as reference drug and treated similarly for determination of absorbance. This procedure was repeated for both the n hexane and ethylacetate fractions.

$$\%\ \text{inhibition} = 100 \times ([Vt/Vc] - 1).$$

Where Vt = absorbance of test sample

Vc = absorbance of control.

2.2.8 Evaluation of Analgesic Activity of the Extract

2.2.8.1 Acetic Acid Induced Writhing Method

The method of Arunachalam *et al.* (2009) was used to determine the analgesic activity of 20% methanol fraction of *Cola hispida* extract. Twenty (20) male albino rats were divided into five groups with each consisting of four animals. Three groups were given different dose of the plant fraction, while the control group was given the vehicle (normal saline) and the reference group was given 5 mg/kg b.w of indomethacin just one hour before 0.6% acetic acid 10 ml/kg, intraperetoneal (I.P) administration. Five minutes after the acetic acid injection i.p., the number of writhes was counted to determine analgesic activity of *Cola hispida* methanol fraction. The animals were placed in a glass jar individually and the contractions of abdominal muscles together with stretching of the hind limbs were cumulatively counted over a period of 30 minutes. Percent inhibition of pain for each group was calculated by comparing the total writhetic number of writhes in the group over the 30 minutes period with the number of writhes in the control group over the same time period. Data were calculated according to the following formula.

The percentage protection against writhing was taken as an index of analgesia and calculated using the following formula,

$$\%\ \text{Analgesic Activity} = \frac{\text{Mean writhing count (control group –treated group)} X\ 100}{\text{Mean writhing count of control group}}$$

2.2.8.2 Formalin Induced Nociception

The method described by Badilla *et al.* (2003) was used for the formalin induced nociception. Nociception was induced by injecting 0.1ml of 2.5% formalin into the plantar surface of the left hind-paw of the rat 60mins after treatment with the extract. The test was carried out in a transparent plastic chamber (30 x 30 x 30) cm with a mirror placed at the base (bottom) of the chamber to allow an unobstructed view of the rats. The time that the animal spent licking the injected paws was measured as an index of pain or nociception. The initial nociceptive response after formalin injection was also taken into account. Twenty (20) male albino rats were divided into five groups with each consisting of four animals. Three groups were given different dose of the plant fraction, while the control group was given the vehicle (normal saline) and the reference group was given 5 mg/kg b.w of indomethacin just one hour before the formalin induced pain.

2.2.9 Evaluation of Anti-inflammatory Activities

2.2.9.1 Egg Albumin Induced Inflammation

The anti-inflammatory activities of *Cola hispida* fraction was determined using the methods described by Arunachalam *et al.* (2009). The test was carried out using a phlogistic agent – induced mouse hind paw oedema as a model of acute inflammation. The phlogistic agent employed in this study was fresh egg-albumin (Akah and Nwambie, 1994). Adult male albino rats (83 - 124g) were used after a 12 hr fast. Animals were deprived of water only during the experiment. Twenty (20) male albino rats were divided into five groups with each consisting of four animals. Three groups were given different dose of the plant fraction, while the control group was given the vehicle (normal saline) and the reference group was given 5 mg/kg b.w of indomethacin just one hour before inflammation was induced. Inflammation of the hind paw was induced by injection of 0.1ml of fresh egg white into the subplantar surface of the right hind paw of the rats. Paw diameters were measured immediately before the administration of the phlogistic agent and 30 minutes, 1hour, 2 hours, 3 hours and 4 hours thereafter. For routine drug testing, the increase in paw diameter 30 minutes to 4 hours after administration of the phlogistic agent was adopted as the parameter for measuring inflammation. Thus (inflammation) was assessed as the difference between zero time paw diameter and the respective time interval after administration of phlogistic agent (Hess and Milonig, 1972). The fraction (100, 200 and 400 mg/kg) were administered orally 1 hour before inducing inflammation. Control group received equivalent amount of normal

saline and the reference group administered indomethacin 5mg/kg b.w. Average oedema (Ct - Co) and percent inhibition of oedema were calculated for each dose (Akah and Nwambie, 1994).

2.2.9.2 Evaluation of Effect of the Fraction on Agar-Induced Leukocyte Mobilization in Male Albino Rats

The method described by Rebeiro *et al.* (1991) was used for the evaluation of effect of the fraction on agar-induced leukocyte mobilization in male albino rats. Twenty adult male albino rats were divided into five groups of four animals each. The groupings are a replica of groupings under the paw oedema. Three groups recieved different dose of the plant fraction, while the control group was given the vehicle (normal saline) and the reference group was given 5 mg/kg b.w of indomethacin. One hour after oral administration of the drugs, each rat in the various groups received intraperitoneal injections of 0.5ml of 3% w/v agar suspension in distilled water. Four hours later the rats were sacrificed and the peritoneum was washed with 5ml of 5% solution of EDTA in phosphate buffered saline. The peritoneal fluid was recovered. Total leukocyte count (TLC) and differential leukocyte count (DLC) were determined on the peritoneal fluid.

2.2.10 Statistical Analysis

The data obtained were analysed using IBM Statistical Product and Service Solution (SPSS) version 20.0 and microsoft excel 2010. The result was presented as Mean ± standard deviation and confidence interval $p < 0.05$ of the result were considered.

CHAPTER THREE

RESULTS

3.1 Percentage Yield and Phytoconstituents of *Cola Hispida*

The extraction of *Cola hispida* leaf using methanol gave a yield of 72g which represented a percentage yield of 7.2%. The phytochemical analysis shows the presence of flavonoids (4.28 ± 0.28 mg/g), phenolic compounds (4.03 ± 0.03 mg/g), Tanins (1.27 ± 0.04 mg/g), Alkaloids (1.68 ± 0.02 mg/g), Glycosides (0.84 ± 0.02 mg/g) and Saponins (0.74 ± 0.06 mg/g). However, Terpenoid was not detected. Table 1 shows the phytochemical constituent of *Cola hispida*.

3.2 Acute Toxicity Studies

The result of acute toxicity studies shows that there was no mortality at the dose of 10, 100 and 1000 mg/kg b.w respectively. This is phase I of the acute toxicity studies. In phase II of the acute toxicity studies, one animal died in the groups that received 1600 and 2900 mg/kg b.w respectively. Two rats died in the group that received the highest dose (5000 mg/kg b.w).

Table 1: Phytoconstituents of *Cola hispida* Methanol Extract

Phytoconstituents	Abundance	Amount (%)
Flavonoids	+++	4.28±0.28
Phenolic Compounds	++	4.03±0.03
Tanins	++	1.27±0.04
Alkaloids	+	1.68±0.02
Glycosides	+	0.84±0.02
Saponins	+	0.74±0.06
Terpenoids	Not detected	Not detected

Values are expressed as Mean±SD. n=3

Key

+++ = Highly present

++ = Moderately present

+ = Slightly present

Table 2: Acute Toxicity Studies

Groups	Number of animals	Mortality
PHASE I		
10 mg/kg b.w Extract	3	0/3
100 mg/kg b.w Extract	3	0/3
1000 mg/kg b.w Extract	3	0/3
PHASE II		
1600 mg/kg b.w Extract	3	1/3
2900 mg/kg b.w Extract	3	1/3
5000 mg/kg b.w Extract	3	2/3

n = 3

3.3 *In Vitro* Anti-inflammatory Activity of *Cola hispida*

The *in vitro* anti-inflammatory activity evaluation of indomethacin, 20% methanol fraction, n hexane fraction and ethylacetate fraction showed that at the concentration, 100 µg/ml, the percentage inhibition of indomethacin was higher than those of 20% methanol fraction, n hexane fraction and ethylacetate fraction while the percentage inhibition of 20% methanol fraction was higher than those of n hexane and ethylacetate fractions respectively while the percentage inhibition of protein denaturation of ethylacetate fraction was higher than that of n-hexane. At the concentration, 200 µg/ml, the percentage inhibition of indomethacin was higher than those of 20% methanol fraction, n hexane fraction and ethylacetate fraction while the percentage inhibition of 20% methanol fraction was higher than those of n hexane and ethylacetate fractions respectively while the percentage inhibition of protein denaturation of ethylacetate fraction was also found to be higher than that of n hexane.

At the concentration, 300 µg/ml, the percentage inhibition of indomethacin was higher than those of 20% methanol fraction, n hexane fraction and ethylacetate fraction while the percentage inhibition of 20% methanol fraction was higher than those of n hexane and ethylacetate fractions respectively while the percentage inhibition of protein denaturation of ethylacetate fraction was also seen to be higher than that of n hexane. This was also observed for the concentrations, 400 µg/ml and 500 µg/ml respectively.

Table 3: *In vitro* Anti-inflammatory Activity

		% Inhibition of Protein Denaturation			
Group	Concentration (µg/ml)	Indomethacin	20% Methanol Fraction	n-Hexane Fraction	Ethylacetate Fraction
Group1	100	12.93 ± 5.74	5.43 ± 0.82	3.55 ± 1.42	4.49 ± 0.81
Group2	200	36.10 ± 4.92	14.89 ± 1.23	12.05 ± 5.63	4.95 ± 1.25
Group3	300	42.01 ± 3.63	41.30 ± 4.09	17.96 ± 1.47	11.11 ± 0.81
Group4	400	60.69 ± 9.66	54.06 ± 4.03	45.32±12.82	21.52 ± 0.52
Group5	500	72.31 ± 12.09	61.15 ± 4.09	57.85 ± 1.63	39.96 ± 0.82

Values are presented as mean SD. n=3.

3.4 Effect of 20% Methanol fraction of *Cola hispida* leaf on egg albumin induced Paw oedema

The result of the effect of 20% methanol fraction of *Cola hispida* leaf on egg albumin induced inflammation at different time intervals showed that there was a significant ($p < 0.05$) decrease in the paw size of groups that received the standard drug, indomethacin and 400 mg/kg b.w 20% fraction respectively after thirty (30) minutes of induction. However there was a non-significant ($p > 0.05$) decrease in the paw size of groups 4 and 5 animals that received 100 and 200 mg/kg b.w 20% fraction respectively, compared to the positive control after 30 minutes of egg albumin induced inflammation. The effect of 20% methanol fraction on egg albumin induced inflammation in the experimental animals after one hour of induction showed that there was a significant ($p < 0.05$) decrease in the paw sizes of the groups that received the standard drug, indomethacin and graded doses of the 20% methanol fraction compared to the positive control. However, a dose dependent significant ($p < 0.05$) decrease in paw sizes of experimental animals were observed as the dose of the 20% methanol fraction increased from 100 to 400 mg/kg b.w. The effect of 20% methanol fraction on egg albumin induced inflammation in the experimental animals after two hours of induction showed that there was a significant ($p < 0.05$) decrease in the paw sizes of the groups that received the standard drug, indomethacin and graded doses of the 20% methanol fraction compared to the positive control. However, a dose dependent significant ($p < 0.05$) decrease in paw sizes of experimental animals were observed as the dose of the 20% methanol fraction increased from 100 to 400 mg/kg b.w. The effect of 20% methanol fraction on egg albumin induced inflammation in the experimental animals after three hours of induction showed that there was a significant ($p < 0.05$) decrease in the paw sizes of the groups that received the standard drug, indomethacin and graded doses of the 20% methanol fraction compared to the positive control. On the other hand, a dose dependent significant ($p < 0.05$) decrease in paw sizes of experimental animals were observed as the dose of the 20% methanol fraction increased from 100 to 400 mg/kg b.w. After four(4) hours of induction, a significant ($p < 0.05$) increase in paw size was observed in the positive control group compared to the groups that received the standard drug, indomethacin and graded doses of the 20% methanol fraction. Though, there was a non-significant decrease in the paw size of the group that received the standard drug, indomethacin when compared to the groups that received the graded doses of 20% methanol fraction.

Table 4: Effect of 20% Methanol fraction of Cola hispida leaf on egg albumin induced Paw oedema

	Difference in Paw Size and % Inhibition				
Groups	0.5 hrs	1 hr	2 hrs	3 hrs	4hrs
Group 1	1.10 ± 0.08^{b}	1.15 ± 0.05^{c}	0.87 ± 0.05^{c}	0.72 ± 0.12^{c}	0.60 ± 0.08^{ac}
Group 2	0.55 ± 0.17^{a}	0.52 ± 0.12^{a}	0.40 ± 0.08^{a}	0.25 ± 0.05^{a}	0.22 ± 0.05^{a}
	(50.00%)	(54.78%)	(54.02%)	(65.27%)	(63.33%)
Group 3	0.92 ± 0.09^{b}	0.95 ± 0.05^{c}	0.62 ± 0.12^{ab}	0.37 ± 0.09^{ab}	0.32 ± 0.05^{a}
	(16.36%)	(17.39%)	(15.73%)	(48.61%)	(46.66%)
Group 4	0.95 ± 0.30^{b}	1.00 ± 0.31^{bc}	0.72 ± 0.22^{bc}	0.47 ± 0.18^{b}	0.32 ± 0.09^{a}
	(13.63%)	(13.04%)	(17.24%)	(34.72%)	(46.66%)
Group 5	0.67 ± 0.05^{a}	0.75 ± 0.05^{b}	0.55 ± 0.12^{ab}	0.32 ± 0.09^{ab}	0.30 ± 0.08^{a}
	(39.09%)	(34.78%)	(36.78%)	(55.55%)	(50.00%)

Values are expressed as Mean ± SD, n=4. Values in the same column having different superscripts differ significantly ($p < 0.05$). Group 1: Positive control; Group 2 = 5mg/kg b.w indomethacin; Group 3: 100mg/kg b.w 20% methanol fraction; Group 4: 200mg/kg b.w 20% methanol fraction; Group 5: 400mg/kg b.w. 20% methanol fraction.

3.5 Effect of 20% Methanol fraction of *Cola hispida* leaf on acetic acid induced Writhing

Table 5 shows the result of the effect of 20% methanol fraction on acetic acid writhing showed that there was a significant ($p < 0.05$) increase in the number of writhes in the positive control group that received intraperitoneal dose of acetic acid compared to the standard drug control (Indomethacin) and groups that received the graded doses of the fraction. There were significant ($p < 0.05$) decrease in the number of writhes in the groups that received 200 and 400 mg/kg b.w 20% fraction compared to the group that received 100 mg/kg b.w 20% fraction. However, there was a non-significant ($p > 0.05$) decrease in the number of writhe in the group that received 400 mg/kg 20% fraction b.w compared to the group that received 200mg/kg b.w 20% fraction. In addition, there was a significant ($p < 0.05$) decrease in the number of writhes in the group that received the indomethacin.

3.6 Effect of 20% Methanol fraction of *Cola hispida* leaf on formalin induced nociception

Table 6 shows the effect of 20% methanol fraction on formalin induced paw licking in rats showed a significant ($p < 0.05$) decrease in the paw licking time in the group that received the standard drug and groups that received the graded doses of the fraction compared to the untreated group that received formalin in the hind limb only. There were significant ($p < 0.05$) decrease in paw licking time in the groups that received 200 and 400mg/kg b.w 20% fraction compared to the group that received 100 mg/kg b.w 20% fraction. However, there was a non-significant ($p > 0.05$) decrease in paw licking time in the group that received 400 mg/kg 20% fraction b.w compared to the group that received 200 mg/kg b.w 20% fraction. In addition, there was a significant ($p < 0.05$) decrease in paw licking time in the group that received the indomethacin.

Table 5: Effect of 20% Methanol fraction of *Cola hispida* leaf on acetic acid induced Writhing

Group	Number of Writhe	% Inhibition
Group 1	38.75 ± 2.21^{d}	-
Group 2	10.00 ± 1.41^{a}	74.19
Group 3	22.75 ± 3.20^{c}	41.29
Group 4	17.00 ± 2.58^{b}	56.12
Group 5	17.50 ± 1.91^{b}	54.83

Values are expressed as mean ± SD, n=4. Values in the same column having different superscripts differ significantly ($p < 0.05$). Group 1: Positive control; Group 2 = 5mg/kg b.w indomethacin; Group 3: 100mg/kg b.w 20% methanol fraction; Group 4: 200mg/kg b.w 20% methanol fraction; Group 5: 400mg/kg b.w. 20% methanol fraction.

Table 6: ffect of 20% Methanol fraction of *Cola hispida* leaf on formalin induced nociception

Group	Time(secs)	% Inhibition
Group 1	35.00 ± 3.82^{d}	-
Group 2	7.25 ± 1.50^{a}	79.28
Group 3	19.75 ± 3.30^{c}	43.57
Group 4	12.25 ± 2.06^{b}	65.00
Group 5	10.50 ± 2.51^{ab}	71.42

Values are expressed as mean ± SD, n=4. Values in the same column having different superscripts differ significantly ($p < 0.05$). Group 1: Positive control; Group 2 = 5mg/kg b.w indomethacin; Group 3: 100mg/kg b.w 20% methanol fraction; Group 4: 200mg/kg b.w 20% methanol fraction; Group 5: 400mg/kg b.w. 20% methanol fraction.

3.7 Effect of 20% Methanol fraction of *Cola hispida* leaf on agar induced leukocyte mobilization

Table 7 shows the effect of lincomycin on agar induced leukocyte mobilization in rats. There was a decrease in agar induced mobilization of leukocytes upon treatment with 20% methanol fraction of Cola hispda leaf. This decrease was dose dependent with the plant fraction 100mg/kg, 200mg/kg and 400mg/kg having total leukocyte counts of 82.02, 76.48 and 64.50 (10^4) respestively. Neutrophils were the most mobilized leukocytes. Basophils were barely mobilized. The total cell count was lower in indomethacin treated group when compared to values obtained for all doses of the plant fraction treated rats. The difference between the value obtained for indomethacin treated group and that of the rats treated with the highest dose of the plant fraction was non-significant ($p > 0.05$).

Table 7: Effect of 20% Methanol fraction of *Cola hispida* leaf on Agar induced leukocyte mobilization

Groups	TLC[+10$^{)4}$]	% Inhibition	Nuetrophils	Lymphocytes	Monocytes	Eosinophils
Group 1	96.02 ± 15.04^{d}	-	65.18 ± 6.55	32.20± 4.32	1.10 ± 0.01	0.91 ± 0.1
Group 2	58.23 ± 4.84^{a}	39.35	61.80 ± 4.02	35.00 ± 3.20	1.50 ± 0.10	1.70 ± 0.1
Group 3	82.02 ± 7.02^{c}	14.58	61.73 ± 6.20	35.06 ± 2.38	1.60±0.010	1.72 ± 0.30
Group 4	76.48 ± 7.28^{b}	20.34	60.10 ± 4.25	37.00 ± 4.61	1.34 ± 0.10	1.63 ±0.01
Group 5	64.50 ± 4.32^{ab}	32.82	57.91	39.21 ± 4.26	1.30 ± 0.20	1.80 ± 0.20

Values are expressed as mean ± SD, n=4. Values in the same column having different superscripts differ significantly ($p < 0.05$).

Key : Group 1: Positive control; Group 2 = 5mg/kg b.w. Indomethacin; Group 3: 100mg/kg b.w. 20% methanol fraction; Group 4: 200mg/kg b.w. 20% methanol fraction; Group 5: 400mg/kg b.w. 20% methanol fraction.

CHAPTER FOUR

DISCUSSION

In this study, the anti-inflammatory activity of *Cola hispida* leaves and their ability to reduce the production of pro-inflammatory mediators have been investigated both *in vivo* and *in vitro*. The results of phytochemical evaluation of *Cola hispida* leaves showed the presence of alkaloid, flavonoid, glycosides, phenolic compounds, saponin and tannin. Bioactive compounds stored in plants possess biological activities that can be used for therapeutic purposes. Flavonoids are considered as health promoting and disease preventing dietary supplements, like other phytonutrients, flavonoids are powerful antioxidants with anti-inflammatory and immune system benefits (Zhang and Reddy, 2018). Tannins are also one of the many phytochemicals that has various pharmacological activities against many chronic diseases such as cardiovascular disease, inflammatory diseases, cancer, obesity and diabetes due to their high antioxidant activity (Kumari and Jain, 2012). This result also agreed with previous research works that was carried out on *Cola millenii* specie close to the *Cola hispida* sterculiaceae family (Faith and Bolurunduro, 2018).

Acute toxicity test accesses the adverse effects that occur within a short time after administration of a single dose of a test substance. This testing is performed principally in rodents and is usually done early in the development of a new chemical or product to provide information on its potential toxicity (Yeboah and Osafo, 2017). For acute toxicity study, methanol extract of *Cola hispida* leaves were given to the mice orally at gradual doses of 10 mg/kg, 100 mg/kg, 1,000 mg/kg, 1,600 mg/kg, which is phase I of the acute toxicity studies. In phase II, 2,900 mg/kg and 5,000 mg/kg b.w. were administered and were closely monitored for 24 hours. The groups in phase I did not produce any signs of toxicity at the three doses employed. No mortality was observed in the treated groups in the phase I reaction during the study period. one animal died in the groups that received 1600 and 2900 mg/kg b.w respectively. Two rats died in the group that received the highest dose 5000 mg/kg b.w. Therefore, the LD_{50} of the extract could be lower than 1600 mg/kg. The methanol extract may, therefore, be considered not to be relatively safe on acute exposure. The present finding from the acute toxicity study agreed with the study reported by other researchers that there was toxic nature of the methanol leaf extract of *Cola millenii* up to 1600 mg/kg (Joseph *et al.*, 2017). In addition, the

study done on methanol extract of *Cola millenii* by Joseph *et al.*, (2017) showed that there was no sign of toxicity and mortality at 1000 mg/kg dose.

Table 3 shows the percentage inhibition of protein denaturation by the different fractions of *Cola hispida* leaf extract. Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of the plant fractions to inhibit protein denaturation was studied. This was also used to determine which of the three fractions (20% methanol fraction, n hexane fraction and ethylacetate fraction) is most effective. 20% methanol fraction was the most effective in inhibiting heat induced albumin denaturation, maximum inhibition of 61.15% was observed at 500 µg/ml. Indomethacin, a standard anti-inflammatory drug showed the maximum inhibition at 72.31% at the concentration of 500 µg/ml compared with the control (Table 3). Each value represents the mean ± SD. n=3, Experimental group were compared with control, $p < 0.05$, was considered significant. Membrane stabilization has been used as a method to study the *in vitro* anti-inflammatory activity because the protein membrane is analogous to the lysosomal membrane (Ghildyal *et al.*, 2010), and its stabilization implies that the fraction may well stabilize lysosomal membranes. Stabilization of lysosome is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane (Bennett *et al.*, 2018).

Egg albumin-induced rat paw oedema and agar-induced leukocyte mobilization to the intraperitoneal cavity were the two models used for acute inflammation and the inflammatory reaction was induced in normal animals. The plant fraction was administered orally, 1 hour before injecting the philogistic agent. Egg albumin induction of inflammation involves two distinct phases of mediators release including histamine, kinnins and 5-hydroxytryptamine in the first phase, and prostaglandin in the second phase (Yofen *et al.*, 2015). Prostaglandin in particular, is known to

cause or enhance the cardinal signs of inflammation (Bhadrapura *et al*., 2018) and also partly due to the trauma of the injection. Drugs that inhibit egg albumin-induced paw oedema may act through inhibition of leukocyte migration and prostaglandin synthesis (Sakat *et al*., 2010). In the egg albumin-induced oedema, the extract (100–400 mg/kg b.w) was observed to have exerted significant ($p < 0.05$) effect at the early stage of inflammation (1–2 hours) indicating effect probably on histamine, serotonin and kinnins that are involved in the early stage of egg albumin induced oedema (Sudharshan, 2016). The extract further significantly ($p < 0.05$) inhibited later stage of the oedema maybe due to its ability to inhibit prostaglandin which is known to mediate the second phase of egg albumin induced inflammation (Sakat *et al*., 2010). However, indomethacin (5 mg/kg b.w) a prototype NSAID, a cyclooxygenase inhibitor whose mechanism of action involves inhibition of prostaglandin, inhibited significantly ($p < 0.05$) paw swelling due to egg albumin injection. Flavonoids are reported to be involved in antiinflammatory activity of plants (Moghadmtousi *et al.*, 2015). These have been found to be present in the plant fraction.

From the result in Table 7, there was a dose dependent significant ($p < 0.05$) decrease in leucocyte mobilization upon treatment with all three doses of the plant fraction. The agar suspension was able to cause an injury which was responded to by the test drug, probably suppressing the proliferation of inflammatory mediators that could cause an increase in the production of leucocytes that could migrate to the area. Of the various leucocytes mobilized, neutrophils were the most abundant. The number of neutrophils and other phagocytic cells increased during injury and were directly responsible for the high number of leukocytes that were mobilized to the intraperitoneal cavity (Ior, 2012). These neutrophils attack and decimate the cause of infection using lactoferrin, gelatinase and myeloperoxidase present in granules. They degrade the extracellular matrix, digest the phagocytosed material and bring about inflammation (Ghildiyal *et al.*, 2010). Indomethacin used in this study decreased the mobilization of leukocytes and this is in line with Oukacha *et al* (2018) that stated that high doses of indomethacin inhibited the accumulation of leukocytes.

Formalin induced-paw licking and acetic acid injection to the intraperitoneal cavity were the two models used to test for the analgesic activity of the plant fraction and the nociceptive reaction was induced in normal animals. The plant fraction significantly ($p < 0.05$) reduced acetic acid-induced writhing, formalin-induced hind paw licking as well as reduced the duration of animals of the pain. Acetic acid is a synthetic carboxylic acid with antibacterial and antifungal properties. Although its

mechanism of action is not fully known, undissociated acetic acid may enhance lipid solubility allowing increased fatty acid accumulation on the cell membrane or in other cell wall structures leading to the activation of phospholipase A_2 which synthesizes arachidonic acid, a precursor for prostaglandin synthesis by the enzyme cyclooxygenase. Acetic acid causes inflammatory pain by inducing capillary permeability and in part through local peritoneal receptors from peritoneal fluid concentration of prostaglandin E_2 (PGE_2) and Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Arbab, 2011). This test alone cannot specify the involvement of either central or peripheral activity (Joen et al., 2019). Thus, formalin tests are usually carried out in addition to the above to distinguish between peripheral and central pain. Centrally acting drugs inhibit both abdominal constriction test and peripheral tests (Buer, 2014), while the peripherally acting drugs inhibit only the abdominal constriction (Buer, 2014).

Formalin exhibits neurogenic and inflammatory pains (Kaur *et al.*, 2014) and measures both centrally and peripherally mediated activities that are characteristics of biphasic pain response. The injection of formalin has been reported to cause an immediate and intense increase in the spontaneous activity of Carbon fiber afferent and evoke a distinct quantifiable behavior indicative of pain demonstrated in paw licking by the animals (Kaur *et al.*, 2014). The antinociceptive activities exerted by this extract may be attributed to the presence of secondary metabolites like saponins, flavonoids, tannins, and terpenes. Flavonoids also have anti-inflammatory effects through its inhibition of the cyclooxygenase pathway (Verma *et al.* 2014). That the extract inhibited neurogenic and non-neurogenic pains as well as narcotic pains may in part explain the mechanisms of its action and these effects are due to the presence of phytochemical components in the plant fraction.

4.2 CONCLUSION

The result of the present study has shown that 20% methanol fraction of *Cola hispida* leaf posseses anti-inflammatory and analgesic effects. The results also showed that the anti-inflammatory and analgesic effect were dose dependent and is comparable to the 5 mg/kg b.w indomethacin used as the standard drug. The anti-inflammatory activity, probably depends on the ability of the plant fraction to prevent the production of some pro-inflammatory mediators and cytokines. The highest dose of the plant fraction which is 400mg/kg b.w had the highest effect on the paw oedema, anti-

nonciceptive and leukocyte mobilization tests. The plant fraction inhibited paw oedema especially at the second stage (from 3 hours after induction). All three doses of the plant fraction prevented leukocyte mobilization. Despite the fact that here were non-significant differences between the maximum dose effects of the test drugs and the reference drugs, these test drugs actually have these effects to an extent.

4.3 SUGGESTIONS FOR FURTHER STUDIES

Utilizing the therapeutic potentials from this plant fraction through further investigation is being advocated especially in elucidating cellular mechanisms and establishing structural components of the active ingredients with a view of standardizing them.

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