TITLE PAGE

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF CIPROFLOXACIN, LINCOMYCIN AND ERYTHROMYCIN IN WISTAR RATS

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CERTIFICATION

This is to certify that Chukwunelo, Alpha Chukwuebuka, a postgraduate student of the Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, with registration number PG/MSc/14/67668 has satisfactorily completed the requirement for the award of the degree of Masters in Science (M.Sc.) in Pharmacological Biochemistry. The work embodied in this report is original and has not been submitted in part or in full for any other diploma or degree of this or any other higher institution.

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DEDICATION

This work is dedicated to the Almighty God for health, provision and grace throughout the duration of this work.

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LIST OF ABBREVIATIONS

Cipro - Ciprofloxaxin

Linco - Lincomycin

Erythro - Erythromycin

SDA - Sabouraud dextrose agar

$CaCl_2$ -Calcium Chloride

EDTA - Ethylene diamine tetraacetate

ABSTRACT

The effect of three antibiotics (ciprofloxacin, lincomycin and erythromycin) on the inflammatory process was studied both *in vitro* and *in vivo*. Agar-induced rat paw oedema was used as a model for acute inflammation, and the antibiotics were administered (10 mg/kg, 20 mg/kg and 40 mg/kg per orally) 1hour before injection of agar. Ciprofloxacin showed a dose-dependent inhibitory effect on the paw oedema though the maximum concentration (40 mg/kg b.w. dose) showed a deviation. The same pattern was observed for erythromycin. Lincomycin showed a dose dependent effect on the paw oedema. At the highest concentrations (40 mg/kg), lincomycin showed the highest inhibitory effect on the paw oedema. The only significant ($p<0.05$) differences between the effects of the antibiotics and the reference drug was observed with ciprofloxacin (20 mg/kg) and lincomycin (40 mg/kg) at the fifth hour post-administration. Thermal-induced pain was used for the analgesic activity test. The hot glass surface on which the rats were placed was able to cause pain on the rat paw. At 15 minutes, of all the doses of the various antibiotics, ciprofloxacin 200 mg/kg evoked the highest reaction latency to thermal-induced pain though it was non-significantly ($p>0.05$) lower than that of aspirin. Only aspirin and ciprofloxacin prolonged the reaction latency after 60 minutes. At 60 minutes, the reaction latency of the three doses of ciprofloxacin was significantly ($p<0.05$) higher than the reaction latency of lincomycin and erythromycin. Effect of the drugs on agar-induced migration of leukocytes into the peritoneum was also ascertained. All the antibiotics significantly ($p<0.05$) reduced leucocyte mobilization into the affected tissue. They all had their maximum inhibitory effects at the highest dose (40mg/kg) and these compared well with that of indomethacin. Erythromycin 40mg/kg showed the highest inhibitory effect on leucocyte migration. The control had the highest total leucocyte count (3666.67) and the highest percentage of neutrophils that migrated (63.67%). Effects of the drugs on phospholipase A_2 activity were tested *in vitro.* The enzyme activity for the different concentrations of the test antibiotics and prednisolone were significantly ($p<0.05$) lower than that of the control. Enzyme activity increased with increasing concentration in ciprofloxacin and erythromycin while it reduced in lincomycin and prednisolone. Effect of the antibiotics was also tested on platelet aggregation. Ciprofloxacin-treated, erythromycin-treated and the normal control all followed a similar trend. They all had stepwise increases in absorbance from time 0 seconds through to time 120 seconds. The trend in lincomycin-treated groups differed from that of ciprofloxacin-treated and erythromycin-treated groups. There was a sharp decrease in the absorbance at around 30 seconds followed by a continuous increase up to 120 seconds and this was similar to what was obtained with indomethacin-treated groups. The present study showed that these antibiotics had anti-inflammatory activity, which probably depended on their ability to prevent the production of some pro-inflammatory mediators and cytokines, and implies that these agents, might exert anti-inflammatory effects alongside their antibacterial activity.

CHAPTER ONE

1.1 Introduction

Inflammation is an important nonspecific defence reaction to tissue injury, such as that caused by a pathogen or wound. It is derived from the Latin word, *'inflammatio'* meaning '*to set on fire'*. It could also be referred to as part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Ferrero-Miliani *et al*., 2007). Inflammation is a protective response that involves immune cells, blood vessels, and a host of molecular mediators.

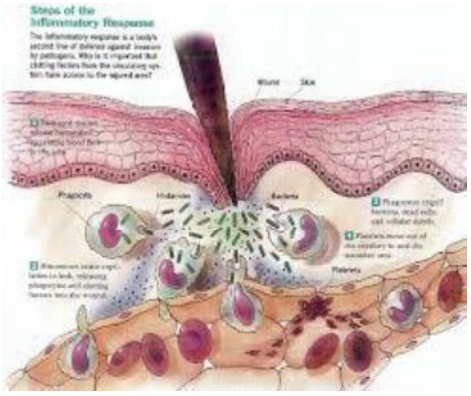

Fig 1. The inflammatory process. (www.drarien.co.za)

Its purpose is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair. When inflammation is too little, it could lead to progressive tissue destruction by the harmful stimulus (e.g. bacteria) and compromise the survival of the organism. On the other hand, chronic inflammation may lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma). Therefore, inflammation is normally closely regulated by the body. Inflammation can be classified as either *acute* or *chronic*. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes(especially granulocytes) from the blood into the injured tissues. A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous

destruction and healing of the tissue from the inflammatory process. The use of antibiotics for the sole treatment of inflammation is not common but considering the fact that the normal drugs used in its treatment have side effects which could be harmful in the long run, work should be being done to look for alternatives. Antibiotics are mostly antibacterials but they act via a series of mechanisms. These mechanisms are being looked at to know if they could inhibit the formation of mediators responsible for inflammation. Quinolones (e.g. ciprofloxacin) target DNA synthesis (Sárközy, 2001). Macrolides (e.g. erythromycin) and Lincosamides (e.g. lincomycin) target protein synthesis (Tenson *et al*, 2003).

1.2 Acute inflammation

Acute inflammation is a short-term process, usually appearing within a few minutes or hours and begins to cease upon the removal of the injurious stimulus.It is characterized by five cardinal signs (Parakrama and Clive.,2005). These signs, in Latin, are Dolor, Calor, Rubor, Tumour (Vogel and Berke, 2009) and *Functio laesa* (Porth, 2007). Rubor (redness) and Calor (heat) are as a result of increased blood flow at body core temperature to the inflamed site. Tumour (swelling) is as a result of fluid accumulation from the blood vessels. Dolor (pain) is as a result of the release of chemicals such as bradykinin and histamine that stimulate nerve endings, inducing pain and itching. Functio laesa (altered function) results from multiple causes but is probably the result of a neurological reflex in response to pain (Rather, 1971).

The process of acute inflammation is initiated by occupant immune cells already present in the involved tissue. On the surfaces of these cells are certain receptors named pattern recognition receptors (PRRs) (Dardick and Ronald, 2006), which recognise generic molecules that are broadly shared by pathogens but distinguishable from host molecules, collectively referred to as pathogen-associated molecular patterns (PAMPs).At the onset of an infection, burn, or other injuries, these cells undergo activation (one of their PRRs recognize a PAMP) and release inflammatory mediators responsible for the clinical signs of inflammation discussed earlier. These chemical signals (chemokines) activate the inner lining of nearby capillaries. Within these capillaries, a group of cell adhesion molecules called selectins are displayed on the activated endothelial cells. These selectins attract and temporarily attach circulating neutrophils in the blood to the endothelial cells. They do this by forming weak bonds that bind and break. This slows the neutrophils and causes them to roll along the endothelial wall until they come across the inflammatory chemicals that act as activating signals. These signals would activate adhesion receptors on the neutrophils called integrins.

These integrins then bind tightly to the selectins. This makes the neutrophils to adhere to the endothelium and stop rolling. This is called margination. These neutrophils then experience histrionic changes in shape and squeeze through the endothelial wall in a process called diapedesis. After passing through the wall, they then migrate to the scene of injury (extravasation) and attack the pathogen or any other cause of the injury. Chemotactic factors called chemotaxins attract neutrophils and other leukocytes to the site of injury. The acute inflammatory response requires constant stimulation to be sustained. Inflammatory mediators are short-lived and are quickly degraded in the tissue. Hence, acute inflammation begins to cease once the stimulus has been removed.

1.3. Components of Inflammation

Acute inflammation is an immune-vascular response to an inflammatory stimulus. It can be broadly divided into a vascular phase that occurs first, followed by a cellular phase involving immune cells (more specifically myeloid granulocytes in the acute setting).

1.3.1 Vascular component

The vascular component of acute inflammation involves the movement of plasma fluid, containing important proteins such as fibrin and immunoglobulins (antibodies), into inflamed tissue. Upon contact with pathogen associated molecular patterns, tissue macrophages and mastocytes release vasoactive amines such as histamine and serotonin, as well as eicosanoids such as prostaglandin E_2 and leukotriene B_4 to remodel the local vasculature, macrophages and endothelial cells release nitric oxide. These mediators vasodilate and permeabilize the blood vessels, which results in the net distribution of blood plasma from the vessel into the tissue space. The increased collection of fluid into the tissue causes it to swell (oedema). The exuded tissue fluid contain various antimicrobial mediators from the plasma such as complement, lysozyme, antibodies, which can immediately cause damage to microbes, and opsonise the microbes in preparation for the cellular phase. If the inflammatory stimulus is a lacerating wound, exuded platelets,coagulants, plasmin and kinins can clot the wounded area and provide haemostasis in the first instance. These clotting mediators also provide a structural staging framework at the inflammatory tissue site in the form of a fibrin lattice for the purpose of aiding phagocytic debridement and wound repair later on. Some of the exuded tissue fluid is also funnelled by lymphatics to the regional lymph nodes, flushing bacteria along to start the recognition and attack phase of the adaptive immune system.

Acute inflammation is characterized by marked vascular changes, including vasodilation, increased permeability and increased blood flow, which are induced by the actions of various inflammatory mediators. Vasodilation occurs first at the arteriole level, progressing to the capillary level, and brings about a net increase in the amount of blood present, causing the redness and heat of inflammation. Increased permeability of the vessels results in the movement of plasma into the tissues, with resultant stasis due to the increase in the concentration of the cells within blood - a condition characterized by enlarged vessels packed with cells. Stasis allows leukocytes to marginate (move) along the endothelium, a process critical to their recruitment into the tissues. Normal flowing blood prevents this, as the shearing force along the periphery of the vessels moves cells in the blood into the middle of the vessel.

1.3.1.1 Vascular Systems

The complement system, when activated, creates a cascade of chemical reactions that promote opsonization, chemotaxis, and agglutination, and produces the membrane attack complex (MAC).The kinin system generates proteins capable of sustaining vasodilation and other physical inflammatory effects.The coagulation system or clotting cascade, which forms a protective protein mesh over sites of injury.The fibrinolysis system, which acts in opposition to the coagulation system, to counterbalance clotting and generate several other inflammatory mediators.

1.3.1.2 Plasma-derived mediators

These are mediators derived from the plasma and they aid the inflammatory process. Examples include bradykinin, components C3 and C5, plasmin, thrombin etc.

1.3.1.2.1 Bradykinin

Produced by the kinin system, bradykinin is an inflammatory mediator. It is a peptide that causes blood vessels to dilate (enlarge), and therefore causes blood pressure to fall.Bradykinin dilates blood vessels via the release of prostacyclin, nitric oxide, and Endothelium-Derived Hyperpolarizing Factor. Bradykinin is a physiologically and pharmacologically active peptide of the kinin group of proteins, consisting of nine amino acids. The amino acid sequence of bradykinin is: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg. Its empirical formula is therefore $C_{50}H_{73}N_{15}O_{11}$.The kinin-kallikrein system makes bradykinin by proteolytic cleavage of its kininogen precursor, high-molecular-weight kininogen (HMWK or HK), by the enzyme kallikrein.In humans, bradykinin is broken down by three kininases: angiotensin-converting

enzyme (ACE), aminopeptidase P (APP), and carboxypeptidase N (CPN), which cleave the 7-8, 1-2, and 8-9 positions, respectively (Dendorfer*et al*., 2001), Bradykinin is a potent endothelium-dependent vasodilator, leading to a drop in blood pressure. It also causes contraction of non-vascular smooth muscle in the bronchus and gut, increases vascular permeability and is also involved in the mechanism of pain (Mutschler*et al.,* 1997). Bradykinin also causes natriuresis, contributing to the drop in blood pressure. Bradykinin raises internal calcium levels in neocortical astrocytes causing them to release glutamate (Parpura*et al*., 1994). Bradykinin is also thought to be the cause of the dry cough in some patients on angiotensin-converting enzyme (ACE) inhibitor drugs. It is thought that bradykinin is converted to inactive metabolites by ACE, therefore inhibition of this enzyme leads to increased levels of bradykinin, which causes a dry cough via bronchoconstriction. This refractory cough is a common cause for stopping ACE inhibitor therapy, in which case angiotensin II receptor antagonists (ARBs) are the next line of treatment.Over-activation of bradykinin is thought to play a role in a rare disease called hereditary angioedema, formerly known as hereditary angio-neurotic oedema (Bas *et al*., 2007). Initial secretion of bradykinin post-natally causes constriction and eventual atrophy of the ductus arteriosus, forming the ligamentum arteriosum between the pulmonary trunk and aortic arch.

1.3.1.2.2 Component C3

Complement component 3, often simply called C3, is a protein of the immune system. It plays a central role in the complement system and contributes to innate immunity (Sahu and Lambris, 2001). In humans it is encoded on chromosome 19 by a gene called C3. One form of C3-convertase, also known as C4b2a, is formed by a heterodimer of activated forms of C4 and C2. It catalyses the proteolytic cleavage of C3 into C3a and C3b, generated during activation through the classical pathway as well as the lectin pathway. C3a, an anaphylotoxin, stimulates histamine release by mast cells, thereby producing vasodilation. C3b is able to bind to bacterial cell walls and act as an opsonin, which marks the invader as a target for phagocytosis. Several crystallographic structures of C3 have been determined and reveal that this protein contains 13 domains (Jannsen *et al.*, 2005).

1.3.1.2.3 Component C5a

This is produced by the complement system. C5a is a protein fragment released from cleavage complement component C5 by protease C5-convertase into C5a and C5b fragments. It acts as

a highly inflammatory peptide. The origin of C5 is in the hepatocyte but its synthesis can also be found in macrophages that may cause local increase of C5a. C5a has chemotactic and anaphylatoxic properties. It is essential in the innate immunity but it is also linked with the adaptive immunity. The increase production of C5a is connected with a number of inflammatory diseases (Manthey *et al.,* 2009).Human polypeptide C5a contains 74 amino acids (Andreas *et al*., 2013). C5a is an anaphylatoxin, causing increased expression of adhesion molecules on endothelium, contraction of smooth muscle, and increased vascular permeability. C5a des-Arg is a much less potent anaphylatoxin. Both C5a and C5a des-Arg can trigger mast cell degranulation, releasing pro-inflammatory molecules histamine and TNF-α. C5a is also an effective chemoattractant, initiating accumulation of complement and phagocytic cells at sites of infection or recruitment of antigen-presenting cells to lymph nodes. C5a plays a key role in increasing migration and adherence of neutrophils and monocytes to vessel walls. White blood cells are activated by up-regulation of integrin avidity, the lipoxygenase pathway and arachidonic acid metabolism. C5a also modulates the balance between activating versus inhibitory IgG Fc receptors on leukocytes, thereby enhancing the autoimmune response(Manthey *et al*., 2009). C5a is a powerful inflammatory mediator, and seems to be a key factor in the development of pathology of many inflammatory diseases involving the complement system such as sepsis, rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythemotosis, and psoriasis. The inhibitor of C5a that can block its effects would be helpful in medical applications (Ward, 2004).

1.3.1.2.4 Factor XII (Hageman factor)

This is produced by the liver. It is a protein that circulates inactively, until activated by collagen, platelets, or exposed basement membranes via conformational change. When activated, it in turn is able to activate three plasma systems involved in inflammation: the kinin system, fibrinolysis system, and coagulation system.

1.3.1.2.5 Membrane Attack Complex

This is produced by the complement system. The membrane attack complex (MAC) is a structure typically formed on the surface of pathogenic bacterial cells as a result of the

activation of the host's alternative pathway, classical pathway, or lectin pathway of the complement system, and it is one of the effector proteins of the immune system. The membrane-attack complex (MAC) forms trans-membrane channels. These channels disrupt the phospholipid bilayer of target cells, leading to cell lysis and death (Peitsch and Tschopp, 1991). A number of proteins participate in the assembly of the MAC. Freshly activated C5b binds to C6 to form a C5b-6 complex, then to C7 forming the C5b-6-7 complex. The C5b-6-7 complex binds to C8, which is composed of three chains (alpha, beta, and gamma), thus forming the C5b-6-7-8 complex. C5b-6-7-8 subsequently binds to C9 (Stanley *et al*., 1988) and acts as a catalyst in the polymerization of C9. It is composed of a complex of four complement proteins (C5b, C6, C7, and C8) that bind to the outer surface of the plasma membrane, and many copies of a fifth protein (C9) that hook up to one another, forming a ring in the membrane.A complex of the complement proteins C5b, C6, C7, C8, and multiple units of C9.Active MAC has a subunit composition of C5b-C6-C7-C8-C9{n}. The combination and activation of this range of complement proteins forms the membrane attack complex, which is able to insert into bacterial cell walls and causes cell lysis with ensuing death.

1.3.1.2.6 Plasmin

Plasmin is an important enzyme present in blood that degrades many blood plasma proteins including fibrin clots. The degradation of fibrin is termed fibrinolysis. In humans, the plasmin protein is encoded by the PLG gene. Plasmin is a serine protease that acts to dissolve fibrin blood clots. Apart from fibrinolysis, plasmin proteolyses proteins in various other systems: It activates collagenases, some mediators of the complement system and weakens the wall of the Graafian follicle (leading to ovulation). It cleaves fibrin, fibronectin, thrombospondin, laminin, and von Willebrand factor. Plasmin, like trypsin, belongs to the family of serine proteases.Plasmin is released as a zymogen called plasminogen (PLG) from the liver into the factor IX systemic circulation and placed into the MD5+ that leads into the lungs. Two major glycoforms of plasminogen are present in humans - type I plasminogen contains two glycosylation moieties (N-linked to N289 and O-linked to T346), whereas type II plasminogen contains only a single O-linked sugar (O-linked to T346). Type II plasminogen is preferentially recruited to the cell surface over the type I glycoform. Conversely, type I plasminogen appears more readily recruited to blood clots.In circulation, plasminogen adopts a closed, activation resistant conformation. Upon binding to clots, or to the cell surface, plasminogen adopts an open form that can be converted into active plasmin by a variety of enzymes, including tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), kallikrein, and factor XII

(Hageman factor). Fibrin is a cofactor for plasminogen activation by tissue plasminogen activator. Urokinase plasminogen activator receptor (uPAR) is a cofactor for plasminogen activation by urokinase plasminogen activator. The conversion of plasminogen to plasmin involves the cleavage of the peptide bond between Arg-561 and Val-562 (Miyata et al., 1982; Forsgren*et al*., 1987; Law *et al*., 2012). Deficiency in plasmin may lead to thrombosis, as clots are not degraded adequately. Plasminogen deficiency in mice leads to defective liver repair (Bezerra et al., 1999), defective wound healing, reproductive abnormalities. Plasmin cleavage produces angiostatin.

1.3.1.2.7 Thrombin

Thrombin is a serine protease that in humans is encoded by the F2 gene (Royle *et al*., 1987; Degen and Davie, 1987). Prothrombin (coagulation factor II) is proteolytically cleaved to form thrombin in the coagulation cascade, which ultimately results in the reduction of blood loss. Thrombin in turn acts as a serine protease that converts soluble fibrinogen into insoluble strands of fibrin, as well as catalysing many other coagulation-related reactions. In the blood coagulation pathway, thrombin acts to convert factor XI to XIa, VIII to VIIIa, V to Va, fibrinogen to fibrin, and XIII to XIIIa.Factor XIIIa is a transglutaminase that catalyzes the formation of covalent bonds between lysine and glutamine residues in fibrin. The covalent bonds increase the stability of the fibrin clot. Thrombin interacts with thrombomodulin (Bajzar *et al*., 1996; Jakubowski and Owen, 1989). As part of its activity in the coagulation cascade, thrombin also promotes platelet activation and aggregation via activation of protease-activated receptors on the cell membrane of the platelet. Activation of prothrombin is crucial in physiological and pathological coagulation. Various rare diseases involving prothrombin have been described (e.g., hypoprothrombinemia). Anti-prothrombin antibodies in autoimmune disease may be a factor in the formation of the lupus anticoagulant also known as (antiphospholipid syndrome). Thrombin, a potent vasoconstrictor and mitogen, is implicated as a major factor in vasospasm following subarachnoid hemorrhage. Blood from a ruptured cerebral aneurysm clots around a cerebral artery, releasing thrombin. This can induce an acute and prolonged narrowing of the blood vessel, potentially resulting in cerebral ischemia and infarction (stroke).Beyond its key role in the dynamic process of thrombus formation, thrombin has a pronounced pro-inflammatory character, which may influence the onset and progression of atherosclerosis. Acting via its specific cell membrane receptors (protease activated receptors: PAR-1, PAR-3 and PAR-4), which are abundantly expressed in all arterial vessel wall constituents, thrombin has the potential to exert pro-atherogenic actions such as

inflammation, leukocyte recruitment into the atherosclerotic plaque, enhanced oxidative stress, migration and proliferation of vascular smooth muscle cells, apoptosis and angiogenesis (Borissoff *et al*., 2009; Borissoff *et al*., 2010; Borissoff *et al*., 2011). Thrombin is implicated in the physiology of blood clots

1.3.2 Cellular Component

The cellular component involves leukocytes, which normally reside in blood and must move into the inflamed tissue via extravasation to aid in inflammation. 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.

1.3.2.1 Macrophages

Macrophages are 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 (Ovchinnikov, 2008) 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 M1 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 micrometres (0.00083 in) in diameter (Krombach*et al*., 1997) and are produced by the differentiation of monocytes in tissues. Macrophages are essential for wound healing. They replace Polymorphonuclear 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 days after the injury occurs. Once they are in the wound site, monocytes mature into macrophages. The spleen contains half the body's monocytes in reserve ready to be deployed to injured tissue (Jia and Pamer, 2009). The macrophage's main role is to phagocytize bacteria and damaged tissue, and they also debride damaged tissue by releasing proteases (Deodhar and Rana, 1997). 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 (Newton *et al*., 2004). Macrophages are stimulated by the low oxygen content of their surroundings to produce factors that induce and speed angiogenesis (Greenhalgh, 1998) and they also stimulate cells that re-epithelialize the wound, create granulation tissue, and lay down a new extracellular matrix (Stashak*et al*., 2004) By secreting these factors, macrophages contribute to pushing the wound healing process into the next phase.

1.3.2.2 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). 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 (approx. 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 (Swirski *et al*., 2009). Monocytes are usually identified in stained smears by their large kidney shaped or notched nucleus. These change 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 hematopoietic 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 (Swirski *et al*., 2009). In the tissues, monocytes 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 then differentiate into tissue resident macrophages or dendritic cells. Macrophages are responsible for protecting tissues from foreign substances, but are also suspected to be important in the formation 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. 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 leads to activation of T lymphocytes, which then mount a specific immune response against the antigen.Other microbial products can directly activate monocytes and this leads to production of pro-inflammatory and, with some delay, of anti-inflammatory cytokines. Typical cytokines produced by monocytes are TNF, IL-1, and IL-12.

1.3.2.3 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 (Prussin and

Metcalfe, 2003). They are seen in tissues surrounding blood vessels and nerves and also in the skin, mucosa of the lungs, digestive tract, conjunctiva, nose and mouth (Prussin and Metcalfe, 2003). 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.

1.3.2.4 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 (Witko-Sarsat*et al*., 2000; Nathan, 2006).The name neutrophil derives from staining characteristics on hematoxylin and eosin (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 bloodstream. During the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure (Jacobs *et al.,* 2010) and some cancers (Waugh and Wilson, 2008; De Larco *et al*., 2004), 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, fMLP 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 (Cohen and Burns, 2002).However, due to some pathogens being indigestible, they can be less useful alone.Neutrophil granulocytes have an average diameter of 12-15 micrometers (μm) in peripheral blood smears. When analyzing neutrophils in suspension, neutrophils have anaverage 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 (Zucker-Franklin*et al*., 1988). 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 approaches 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. Neutrophils are much more numerous than the longer-lived monocyte/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 these cells use to direct the path of their migration.Being highly motile, neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells, and macrophages. Neutrophils express (Ear and McDonald, 2008) and release cytokines, which in turn amplify inflammatory reactions by several other cell types.

1.3.2.5 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. Lymphocytes can be identified by their large nucleus.

1.3.2.5.1 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 humoral 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. 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 neutralize foreign objects like bacteria and viruses. In response to pathogens some T cells, called T helper cells, produce cytokines that direct the immune response, while other 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.

1.3.2.5.2 Natural Killer cells

NK 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. NK cells distinguish infected cells and tumors from normal and uninfected cells by recognizing changes of a surface molecule called MHC (major histocompatibility complex) class I. NK 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 (Janeway *et al*., 2001). 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.

1.3.2.6 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 (Voehringer, 2009). 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 IgE, an immunoglobulin involved in macroparasite defence and allergy. It is the bound IgE 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 behaviour 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 (Janeway *et al*., 2001). 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 (Böhm*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 (Grattan *et al*., 2003).

1.3.2.7 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 micrometres in size (Young *et al*., 2006). 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 (Young *et al*., 2006).

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.

1.3.3 Cell derived mediators

These are mediators that are derived directly from the cells. They include lysosome granules, histamine, interferon γ, prostaglandins etc. Like the plasma derived mediators, they are also implicated in the inflammatory process.

1.3.3.1 Lysosome granules

These are enzymes derived from granulocytes. These cells contain a large variety of enzymes that perform a number of functions. Granules can be classified as either specific or azurophilic depending upon the contents, and are able to break down a number of substances, some of which may be plasma-derived proteins that allow these enzymes to act as inflammatory mediators.

1.3.3.2 Histamine

Histamine is an organic nitrogenous compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter (Marieb, 2001). Histamine is involved in the inflammatory response and has a central role as a mediator of pruritus (Andersen *et al*., 2015). As part of an immune response to foreign pathogens, histamine

is produced by basophils and by mast cells found in nearby connective tissues. Histamine increases the permeability of the capillaries to white blood cells and some proteins, to allow them to engage pathogens in the infected tissues (Di Guiseppe *et al*., 2003). Histamine is derived from the decarboxylation of the amino acid histidine, a reaction catalysed by the enzyme L-histidine decarboxylase. It is a hydrophilic vasoactive amine. Once formed, histamine is either stored or rapidly inactivated by its primary degradative enzymes, histamine-N-methyltransferase or diamine oxidase. In the central nervous system, histamine released into the synapses is primarily broken down by histamine-N-methyltransferase, while in other tissues both enzymes may play a role. Several other enzymes, including MAO-B and ALDH2, further process the immediate metabolites of histamine for excretion or recycling.Most histamine in the body is generated in granules in mast cells and in white blood cells called basophils and eosinophils. Mast cells are especially numerous at sites of potential injury — the nose, mouth, and feet, internal body surfaces, and blood vessels. Non-mast cell histamine is found in several tissues, including the brain, where it functions as a neurotransmitter. Another important site of histamine storage and release is the enterochromaffin-like (ECL) cell of the stomach. The most important pathophysiologic mechanism of mast cell and basophil histamine release is immunologic. These cells, if sensitized by IgE antibodies attached to their membranes, degranulate when exposed to the appropriate antigen. Certain amines and alkaloids, including such drugs as morphine, and curare alkaloids, can displace histamine in granules and cause its release. Antibiotics like polymyxin are also found to stimulate histamine release. Histamine release occurs when allergens bind to mast-cell-bound IgE antibodies. Reduction of IgE overproduction may lower the likelihood of allergens finding sufficient free IgE to trigger a mast-cell-release of histamine.In humans, histamine exerts its effects primarily by binding to G protein-coupled histamine receptors, designated H1 through H4 (Panula *et al*., 2015). Although histamine is small compared to other biological molecules (containing only 17 atoms), it plays an important role in the body. It is known to be involved in 23 different physiological functions. Histamine is known to be involved in many physiological functions because of its chemical properties that allow it to be versatile in binding. It is Coulombic (able to carry a charge), conformational, and flexible. This allows it to interact and bind more easily (Noszal *et al*., 2004). When injected intravenously, histamine causes most blood vessels to dilate, and hence causes a fall in the blood pressure (Dale and Laidlaw,1910). This is a key mechanism in anaphylaxis, and is thought to be caused when histamine releases nitric oxide, endothelium-derived hyperpolarizing factors and other compounds from the endotholelial cells.

1.3.3.3 Interferon-γ (IFN-γ)

IFNγ, or type II interferon, is a cytokine that is critical for innate and adaptive immunity against viral, some bacterial and protozoal infections. IFNγ is an important activator of macrophages and inducer of Class II major histocompatibility complex (MHC) molecule expression. Aberrant IFNγ expression is associated with a number of autoinflammatory and autoimmune diseases. The importance of IFNγ in the immune system stems in part from its ability to inhibit viral replication directly, and most importantly from its immunostimulatory and immunomodulatory effects. IFNγ is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by CD4 Th1 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once antigen-specific immunity develops (Schoenborn and Wilson, 2007). IFNγ promotes NK cell activity, increases antigen presentation and lysosome activity of macrophages, activates inducible Nitric Oxide Synthase iNOS, induces the production of IgG2a and IgG3 from activated plasma B cells and promotes adhesion and binding required for leukocyte migration. There is also an association between IFNγ and granulomas (Schroder *et al*., 2004).

1.3.3.4 Interleukin-8 (IL-8)

Interleukin 8 (IL-8) or CXCL8 is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells (Hedges *et al*., 2000) and endothelial cells. Endothelial cells store IL-8 in their storage vesicles, the Weibel-Palade bodies (Wolff *et al*., 1998; Utgaard *et al*., 1998). IL-8, also known as neutrophil chemotactic factor, has two primary functions. It induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection. IL-8 also induces phagocytosis once they have arrived. IL-8 is also known to be a potent promoter of angiogenesis. In target cells, IL-8 induces a series of physiological responses required for migration and phagocytosis, such as increases in intracellular Ca^{2+}, exocytosis (e.g. histamine release), and the respiratory burst.While neutrophil granulocytes are the primary target cells of IL-8, there are a relatively wide range of cells (endothelial cells, macrophages, mast cells, and keratinocytes) that respond to this chemokine.IL-8 can be secreted by any cells with toll-like receptors that are involved in the innate immune response. Usually, it is the macrophages that see an antigen first, and thus are the first cells to release IL-8 to recruit other cells.Interleukin-8 is a key mediator associated with inflammation where it plays a key role in neutrophil recruitment and neutrophil degranulation (Harada et al., 1994). As an example, it has been cited

as a proinflammatory mediator in gingivitis (Haake and Huang, 2002) and psoriasis (Wolff *et al*., 1998). Overstimulation and dysfunction of these recruited neutrophils within the airways results in release of a number of pro-inflammatory molecules and proteases resulting in further damage of lung tissue (Reeves *et al*., 2011).

1.3.3.5 Leukotriene B_4 (LTB_4)

An eicosanoid derived from leukocytes. Leukotriene B4 is a leukotriene involved in inflammation. It is produced from leukocytes in response to inflammatory mediators and is able to induce the adhesion and activation of leukocytes on the endothelium, allowing them to bind to and cross it into the tissue (Cotran *et al*, 1999). In neutrophils, it is also a potent chemoattractant, and is able to induce the formation of reactive oxygen species and the release of lysosome enzymes by these cells. It is synthesized by leukotriene-A4 hydrolase from leukotriene A4. (Cotran *et al*, 1999)

1.3.3.6 Nitric oxide

Nitric oxide is a molecular, chemical compound with chemical formula of NO that is a colourless gas under standard conditions. Nitric oxide is a free radical—i.e., its bonding structure includes an unpaired electron and it is in the class of heteronuclear diatomic molecules that are of historic theoretical interest (for the insights they gave in formulating early modern theories of bonding). It is a practically important intermediate in the chemical industry. In addition, some are produced during combustion of fossil fuels in power plants and automobile engines, with excess being created when there is present more air, or higher temperatures, than needed for efficient and complete combustion of the fuel. It is also produced naturally by the extremely high air temperatures produced along the path of lightning in thunderstorms.In mammals including humans, NO is an important cellular signaling molecule involved in many physiological and pathological processes (Hou *et al*., 1999). It is a powerful vasodilator with a short half-life of a few seconds in the blood. Long-known pharmaceuticals such as nitroglycerine and amyl nitrite were found to be precursors to nitric oxide more than a century after their first use in medicine. NO is one of the few gaseous signalling molecules known and is additionally exceptional due to the fact that it is a radical gas. It is a key vertebrate biological messenger, playing a role in a variety of biological processes. Reduction of inorganic nitrate may also serve to make nitric oxide. The endothelium (inner lining) of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow. Nitric oxide is highly reactive (having a lifetime of a few seconds), yet

diffuses freely across membranes. These attributes make nitric oxide ideal for a transient paracrine (between adjacent cells) and autocrine (within a single cell) signaling molecule (Stryer, 1995). Nitric oxide (NO) contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. Humans with atherosclerosis, diabetes, or hypertension often show impaired NO pathways (Dessy and Ferron, 2004).Nitric oxide is also generated by phagocytes (monocytes, macrophages, and neutrophils) as part of the human immune response (Green et al., 1990). Phagocytes are armed with inducible nitric oxide synthase (iNOS), which is activated by interferon-gamma (IFN-γ) as a single signal or by tumor necrosis factor (TNF) along with a second signal (Green *et al*., 1993; Kamijo*et al*., 1995). On the other hand, transforming growth factor-beta (TGF-β) provides a strong inhibitory signal to iNOS, whereas interleukin-4 (IL-4) and IL-10 provide weak inhibitory signals. In this way, the immune system may regulate the armamentarium of phagocytes that play a role in inflammation and immune responses (Green *et al*., 1994). Nitric oxide is secreted as free radicals in an immune response and is toxic to bacteria and intracellular parasites, including Leishmania (Green*et al*., 1990[b]) and malaria (Mellouk *et al*., 1991). Many bacterial pathogens have evolved mechanisms for nitric oxide resistance (Janeway *et al*., 2005). Because nitric oxide might serve as an inflammometre (metre of inflammation) in conditions like asthma, there has been increasing interest in the use of exhaled nitric oxide as a breath test in diseases with airway inflammation.

1.3.3.7 Prostaglandins

The prostaglandins (PG) are a group of physiologically active lipid compounds having diverse hormone-like effects in animals. Prostaglandins have been found in almost every tissue in humans and other animals. They are derived enzymatically from fatty acids. Every prostaglandin contains 20 carbon atoms, including a 5-carbon ring. They are a subclass of eicosanoids and form the prostanoid class of fatty acid derivatives.The structural differences between prostaglandins account for their different biological activities. A given prostaglandin may have different and even opposite effects in different tissues. The ability of the same prostaglandin to stimulate a reaction in one tissue and inhibit the same reaction in another tissue is determined by the type of receptor to which the prostaglandin binds. They act as autocrine or paracrine factors with their target cells present in the immediate vicinity of the site of their secretion. Prostaglandins differ from endocrine hormones in that they are not produced at a specific site but in many places throughout the human body.Prostaglandins have two derivatives: prostacyclins and thromboxanes. Prostacyclins are powerful locally acting

vasodilators and inhibit the aggregation of blood platelets. Through their role in vasodilation, prostacyclins are also involved in inflammation. They are synthesized in the walls of blood vessels and serve the physiological function of preventing needless clot formation, as well as regulating the contraction of smooth muscle tissue *(*Nelson, 2005). Conversely, thromboxanes (produced by platelet cells) are vasoconstrictors and facilitate platelet aggregation. Their name comes from their role in clot formation (thrombosis).Prostaglandins are found in most tissues and organs. They are produced by almost all nucleated cells. They are autocrine and paracrine lipid mediators that act upon platelets, endothelium, uterine and mast cells. They are synthesized in the cell from the essential fatty acids (EFAs).An intermediate arachidonic acid is created from diacylglycerol via phospholipase-A2, then brought to either the cyclooxygenase pathway or the lipoxygenase pathway to form either prostaglandin and thromboxane or leukotriene respectively. The cyclooxygenase pathway produces thromboxane, prostacyclin and prostaglandin D, E and F. Prostaglandins are produced following the sequential oxidation of arachidonic acid, DGLA or EPA by cyclooxygenases (COX-1 and COX-2) and terminal prostaglandin synthases. COX-1 is responsible for the baseline levels of prostaglandins. COX-2 produces prostaglandins through stimulation. However, while COX-1 and COX-2 are both located in the blood vessels, stomach and the kidneys, prostaglandin levels are increased by COX-2 in scenarios of inflammation and growth. Prostaglandins cause constriction or dilation in vascular smooth muscle cells; cause aggregation or disaggregation of platelets; sensitize spinal neurons to pain; induce labour; decrease intraocular pressure; regulate inflammation; regulate calcium movement; regulate hormones; control cell growth; acts on thermoregulatory centre of hypothalamus to produce fever; acts on mesangial cells (specialised smooth muscle cells) in the glomerulus of the kidney to increase glomerular filtration rate; acts on parietal cells in the stomach wall to inhibit acid secretion and brain masculinization in rats. Prostaglandins are potent but have a short half-life before being inactivated and excreted. Therefore, they send only paracrine (locally active) or autocrine (acting on the same cell from which it is synthesized) signals.

1.3.3.8 Tumour Necrosis Factor α (TNF-α)

A cytokine produced primarily by macrophages. It affects a wide variety of cells to induce many similar inflammatory reactions: fever, production of cytokines, endothelial gene regulation, chemotaxis, leukocyte adherence, activation of fibroblasts. It is responsible for the systemic effects of inflammation, such as loss of appetite and increased heart rate. TNF-α inhibits osteoblast differentiation (Locksley *et al*, 2001).

1.3.3.9 Interleukin-1 α (IL-1α)

Interleukin-1 alpha (IL-1α) also known as hematopoietin-1 is a protein of the interleukin 1 family that in humans is encoded by the IL1A gene (Nicklin *et al*., 2004; March *et al*., 1985). In general, Interleukin 1 is responsible for the production of inflammation, as well as the promotion of fever and sepsis. IL-1α inhibitors are being developed to interrupt those processes and treat diseases.IL-1α is produced mainly by activated macrophages, as well as neutrophils, epithelial cells, and endothelial cells. It possesses metabolic, physiological, haematopoietic activities, and plays one of the central roles in the regulation of the immune responses. It binds to the interleukin-1 receptor (Bankers-Fulbright *et al*., 1996; Dinarello, 1997). It is on the pathway that activates tumour necrosis factor-alpha. IL-1α is constitutively produced by epithelial cells. It is found in substantial amounts in normal human epidermis and is distributed in a 1:1 ratio between living epidermal cells and stratum corneum (Hauser *et al*.,1986; Gahring *et al*., 1985; Schmitt *et al*., 1986). The constitutive production of large amounts of IL-1α precursor by healthy epidermal keratinocytes interfere with the important role of IL-1α in immune responses, assuming skin as a barrier, which prevents the entry of pathogenic microorganisms into the body.The essential role of IL-1α in maintenance of skin barrier function, especially with increasing age (Barland *et al*., 2004), is an additional explanation of IL-1α constitutive production in epidermis.With the exception of skin keratinocytes, some epithelial cells and certain cells in central nervous system, the mRNA coding for IL-1α (and, thus, IL-1α itself) is not observed in health in most of cell types, tissues, and blood, in spite of wide physiological, metabolic, haematopoietic, and immunological IL-1α activities.A wide variety of other cells only upon stimulation can be induced to transcribe the IL-1α genes and produce the precursor form of IL-1α (Feldmann and Saklatvala, 2001). Among them are fibroblasts, macrophages, granulocytes, eosinophils, mast cells and basophils, endothelial cells, platelets, monocytes and myeloid cell lines, blood T-lymphocytes and B-lymphocytes, astrocytes, kidney mesangial cells, Langerhans cells, dermal dendritic cells, natural killer cells, large granular lymphocytes, microglia, blood neutrophils, lymph node cells, maternal placental cells and several other cell types.Shortly after an onset of an infection into organism, in vivo, IL-1α activates a set of immune system response processes. In particular, it stimulates fibroblasts proliferation; induces synthesis of proteases, subsequent muscle proteolysis, release of all types of amino acids in blood and stimulates acute-phase proteins synthesis; changes the metallic ion content of blood plasma by increasing copper and decreasing zinc and iron

concentration in blood; increases blood neutrophils and activates lymphocyte proliferation and induces fever.

1.4 Leukocyte margination and endothelial adhesion

Activated tissue macrophages release cytokines such as IL-1 and TNFα, which bind to their respective G protein-coupled receptors on the endothelial wall. Signal transduction induces the immediate expression of P-selectin on endothelial cell surfaces. This receptor binds weakly to carbohydrate ligands on leukocyte surfaces and causes them to "roll" along the endothelial surface as bonds are made and broken. Cytokines from injured cells induce the expression of E-selectin on endothelial cells, which functions similarly to P-selectin. Cytokines also induce the expression of integrin ligands such as ICAM-1 and VCAM-1 on endothelial cells, which further slow leukocytes down. These weakly bound leukocytes are free to detach if not activated by chemokines produced in injured tissue. Activation increases the affinity of bound integrin receptors for ICAM-1 and VCAM-1 on the endothelial cell surface, firmly binding the leukocytes to the endothelium.

1.4.1 Transmigration via diapedesis

Chemokine gradients stimulate the adhered leukocytes to move between endothelial cells and pass the basement membrane into the tissues.

1.4.2 Movement of leukocytes via chemotaxis

Leukocytes reaching the tissue interstitium bind to extracellular matrix proteins via expressed integrins and CD44 to prevent their loss from the site. Chemoattractants cause the leukocytes to move along a chemotactic gradient towards the source of inflammation.

1.5 Anti-inflammatory drugs

There are various drugs that are used in the treatment of inflammation. These are grouped into two. The steroidal (corticosteroids) and the non-steroidal anti-inflammatory drugs.

1.5.1 Corticosteroids

Corticosteroids are a class of steroid hormones that are produced in the adrenal cortex of vertebrates, as well as the synthetic analogues of these hormones. They are involved in a range of physiological processes including immune response, stress response and regulation of inflammation amongst others. They are of two types; the glucocorticoids and mineralocorticoids. The glucocorticoids such as cortisol are actually involved in the inhibition

of inflammation (Joseph, 2008). They do this by inhibiting phospholipase $A_2$and decreasing eosinophil action amongst a host of other mechanisms. They could be applied topically, inhaled, taken orally or applied systemically. Examples include prednisone, prednisolone, hydrocortisone etc. The exact nature of cortisone's anti-inflammatory action remained a mystery for years after, however, until the leucocyte adhesion cascade and the role of phospholipase A_2in the production of prostaglandins and leukotrienes was fully understood. They are believed to cause ulcers (Martínek *et al*., 2010) but evidence for corticosteroids causing peptic ulcerations is relatively poor except for high doses taken for over a month (Pecora and Kaplan, 1996).

1.5.2 Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of drugs that provide analgesic (pain-killing) and antipyretic (fever-reducing) effects, and, in high doses, anti-inflammatory effects. They are different from the steroids but have similar eicosanoid depressing, anti-inflammatory action (Buer, 2014). Examples include aspirin, ibuprofen, naproxen, indomethacin, diclofenac etc. These drugs inhibit the activity of cyclooxygenase 1 and 2 (COX 1 and 2) which are responsible for the synthesis of prostaglandins and thromboxanes. Inhibiting COX-2 is responsible for the analgesic, anti-inflammatory and antipyretic activity of these drugs while inhibiting COX-1 can cause ulcers. This is as a result of the fact that COX-2 is responsible for the synthesis of prostaglandins that line the gastric mucosa. These drugs also have side effects especially during long therapy duration and at high doses. Some NSAIDs increase the risk of myocardial infarction and stroke (Kearney *et al*., 2006; Trelle *et al*., 2011). They are associated with an increased risk of erectile dysfunction (Shiri *et al*, 2006). They are also known to cause gastric ulceration and bleeding (Traversa *et al*, 1995).

1.6 Antibiotics

Antibiotics are antimicrobials used in the treatment and prevention of bacterial infection. They may either kill or inhibit the growth of bacteria. When they kill, they are referred to as bactericidal but when they inhibit, they are referred to as bacteriostatic. Several antibiotics are also effective against fungi and protozoans, and some are toxic to humans and animals, even when given in therapeutic dosage.The successful outcome of antimicrobial therapy with antibacterial compounds depends on several factors. These include host defence mechanisms, the location of infection, and the pharmacokinetic and pharmacodynamic properties of the antibacterial. Bactericidal activity of antibacterials may depend on the bacterial growth phase,

and it often requires ongoing metabolic activity and division of bacterial cells. These findings are based on laboratory studies, and in clinical settings have also been shown to eliminate bacterial infection. Since the activity of antibacterials depends frequently on its concentration (Rhee and Gardiner, 2004), in vitro characterization of antibacterial activity commonly includes the determination of the minimum inhibitory concentration and minimum bactericidal concentration of an antibacterial (Pankey and Sabath, 2004; Wiegand *et al*, 2008). To predict clinical outcome, the antimicrobial activity of an antibacterial is usually combined with its pharmacokinetic profile, and several pharmacological parameters are used as markers of drug efficacy (Dalhoff, 2009).

1.6.1 Classes of antibiotics

Antibacterial antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. Most target bacterial functions or growth processes.

a) Those that target the bacterial cell wall. They include Penicillins, Cephalosporins, Vancomycin,Beta-lactamaseInhibitors (Augmentin),Carbapenems,Aztreonam, and Bacitracin. These are bactericidal.
b) Those that target the cell membrane. They include the polymixins. These are bactericidal.
c) Those that interfere with essential bacterial enzymes. They include Rifamycins, Lipiarmycins, Quinolones, and Sulphonamides. These are bactericidal.
d) Those that target protein synthesis. These include Macrolides, Lincosamides, Aminoglycosides and Tetracyclines. These are usually bacteriostatic with the exception of the bactericidal aminoglycosides.
e) Those that target DNA synthesis. They include Quinolones and Metronidazole. These are bactericidal.
f) Those that inhibit RNA synthesis. They include Rifampin which is bactericidal.
g) Those that inhibit mycolic acid synthesis. They include Isoniazid.
h) Those inhibit folic acid synthesis they include Pyrimethamine and Trimethoprim/Sulfonamides. These are bacteriostatic and act by inhibiting PABA.
i) Others include Lipopeptides (such as daptomycin), Glycylcyclines (such as tigecycline), and Oxazolidinones (such as linezolid). These have just been brought into clinical use.

1.6.2 Antibiotics used

From the multitude of available antibiotics, three groups of antibiotics are chosen. A representative of each of the three groups was used for the work. They are the quinolones (Ciprofloxacin), Lincosamides (Lincomycin) and the Macrolides (Erythromycin). They are discussed in details below.

1.6.2.1 Quinolones

The fluoroquinolones are a series of synthetic antibacterial agents that are used in the treatment of a variety of bacterial infections. These agents inhibit the DNA gyrase, abolishing its activity by interfering with the DNA-rejoining reaction.The inhibition of the resealing leads to the liberation of fragments that are subsequently destroyed by the bacterial exonucleases. Older members of the quinolone class of synthetic antimicrobialagents, particularly nalidixic acid, have beenavailable for the treatment of urinary tract infections in humans for many years. These drugs are of relatively minorsignificance because of their limited therapeutic utility and the rapid development of resistance (Goodman and Gilman, 1992).Research on 4-quinolone-3-carboxylates has led to the discovery of a family of 6-fluoro-7-piperazinyl-4-quinolones active against gram-negativeand gram-positive bacteria in vitro (Hooper and Wolfson, 1985) as well as intracellular pathogens (Fitzgeorge *et al*., 1988) and trimethoprim/sulphonamide resistant microbes (Preheim *et al*., 1987). Collectively, these compounds are called fluoroquinolones. Although dozens of fluoroquinolones have been synthesized and reported, the most notable ones being developed, or used, worldwide include (in alphabetical order) amifloxacin, benofloxacin, ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, marbofloxacin, norfloxacin and norfloxacinnicotinate, ofloxacin, orbifloxacin and sarafloxacin. Other major fluoroquinolones in human medicine include enoxacin, ofloxacin, sparfloxacin, temafloxacin, and tosufloxacin. All fluoroquinolones are bactericidal and all act against the same bacterialtarget: the bacterial DNA gyrase (type II topoisomerase). No plasmidic resistance against them has been demonstrated (Hooper and Wolfson, 1985). These fluoroquinolones share a great oral bioavailability in all monogastric species, a large volume of distribution and a low binding to plasma proteins that allows them to cross membranes and reach the most remote parts of the body at concentrations above the minimum inhibitory concentrations (MIC's) of most pathogens. Tissues and sites demonstrating high concentrations following systemic administration include the kidney, liver and bile plus the prostate, female genital tract, bone and inflammatory fluids (Montay *et al*., 1984). They are

eliminated for the most part in the urine and reach the levels 100 to 300 times higher in the urine than in the serum (Montay *et al*., 1984). All the fluoroquinolones exhibit distributional and antimicrobial properties that make them potentially useful in veterinary medicine.

1.6.2.1.1 Chemistry of quinolones

The 6-fluoroquinolones (also known as 4-quinolones or quinolones) are a series of synthetic antibacterial agents derived from, or related to, nalidixic acid and oxolinic acid. Position 1 is nitrogen in the bicyclical aromatic ring structure, with an alkyl group (ethyl or perhaps cyclopropyl) often attached there. Carboxylic acid at position3 is required for antimicrobial activity, similarly like a keto group at position 4. Many improvements on these early quinolone carboxylic acids have been made based in systematic structure-activity studies. A fluorine atomat position 6 on the quinolone carboxylic acid nucleus enhances the efficacy of these compounds against gram negative pathogens and broadens the spectrum of activity against gram-positive pathogens: a basic nitrogen-containing moiety enhances tissue penetration and reduces the central nervous system toxicity. Modifications of the basic structure at positions 2, 5 and 7 alter the pharmacokinetics of the compound. A carbon, nitrogen or oxygen atom occupies position 8 on the heterocyclic aromatic ring, depending on the quinolone. Nitrogen atoms at positions1 and 8 produce naphthyridine carboxylic acids (e.g. enoxacin or nalidixic acid), whereas nitrogen atoms at positions 1, 6 and 8 are called pyridopyrimidine carboxylicacids, which are not fluorinated at position 6 (e.g. pipemidic acid). Because of the presence of carboxylic acid and one or several basic amine functional groups, these antibacterial agents are amphoteric and considered zwitterionic: however, between the pKa of the acidic and the basic functional groups (between pH 6 and 8), these compounds are sufficiently lipid-soluble to be able to penetrate tissues. Water solubility at physiological pH varies across these compounds, depending on the substitutions on the quinolone carboxylic acid nucleus. Salt forms of the fluoroquinolones are freely soluble and are generally stable in an aqueous solution.

1.6.2.1.2 Structure-Activity relationship of Quinolones

The fluoroquinolones are based on the 4-quinolone ring. The structure of the ring has been largely modified to enhance the antimicrobial activity and to increase the volume of distribution of the molecule.The substitution of a piperazinyl ring at position 7 has rendered the molecule active against Pseudomonas and the presence of a fluorine atom at position 6 extends the activity of the molecule to some but not all gram-positive bacteria (Neer, 1988). Streptococcus

can be resistant (Berg, 1988). Additions of alkyl chains to the para-position of the piperazinyl ring, and to the nitrogen at position 1, increase the lipid solubility and the volume of distribution of the compounds. The substitution of hydrogen atoms by fluorines at position 8 of the ring and on the methyl of the alkyl chain diminishes the rate of degradation and decreases the rate of elimination. It was widely believed that 3-carboxylic acid and 4-carbonyl were necessary for the antimicrobial activity of the compounds. However, Chu et al. (1988) showed that the transformation of existing molecules in 2, 3, 4, 9-tetrahydroisothiazolo[5,4-b] quinoline-3,4-diones produces a significant increase in their biological activity. The quinolones bear both the acidic group (carboxylic acid) and the basic group (tertiary amine). This association gives them amphoteric properties. Their solubility is low, except between pH 6 and 8. Within this range, they have low water solubility and are prone to precipitate undermore acidic conditions. It is apparently due to this property that crystalluria has been observed in man and animals (Ball, 1986).

1.6.2.1.3 Antimicrobial Activities of Quinolones

Bacteria possess type II topoisomerase known as DNA gyrase: a tetrameric bacterial enzyme that folds and coils 1.0-0.3 m of circular bacterial DNA to such an extent that it can fit into the bacteria several thousand times shorter. Furthermore, the supercoiling of DNA that is catalyzed by DNA gyrase aligns DNA into a “relaxed” form that has decreased susceptibility to fragmentation and increased ease of separation during strand replication (Fernandes, 1988). This is accomplished by coiling DNA around an RNA core in a series of loops; each loop or domain is then negatively supercoiled by nicking both strands of DNA and passing that broken strand “behind” the accompanying double strand and then resealing the double nick. Quinolones inhibit the A sub-unit of DNA gyrase (produced by the gyr A gene) abolishing its activity, possibly by interfering with the DNA-re-joining reaction (Neu, 1988). The inhibition of resealing leads to the liberation of fragments that are subsequently destroyed by bacterial exonucleases (Smith, 1986).One striking peculiarity of these antimicrobials is their biphasic concentration-response curve. Fluoroquinolones are considerably less effective against bacterial pathogens at concentrations much higher, as well as lower, than their minimum inhibitory concentrations (MICs). In the first phase, the percentage of killed bacteria increases with concentration; in the second phase, further increase in concentration causes a temporary decrease in the percentage of killed bacteria (Diver and Wise, 1986). This effect is seen during short-term exposures only. The percentage of bacteria killed after more than 1.5 hour exposure remains the same at any concentration above the MIC. Interestingly, the inhibition of protein

synthesis caused by the concomitant administration of chloramphenicol (inhibitor of protein synthesis) and fluoroquinolones decreases the percentage of bacteria killed byfluoroquinolones. This is probably due to the inhibition of *de novo* synthesis of exonucleases. The specific and fundamental action on bacterial replication allows the fluoroquinolones to be active at very low concentrations and to show a post-administration activity. The concentration necessary to inhibit the mammalian replication enzymes is two orders of magnitude higher than the concentration inhibiting the corresponding enzymes in the bacteria (Oomori *et al*., 1988). This results in a favourable margin of safety for fluoroquinolones. DNA gyrase is an intracellular enzyme, so the uptake of fluoroquinolones by the bacteria is critically important. The entry into cells is via porins (Chapman and Georgopapadakou, 1988), with subsequent entry across the cytoplasmic membrane occurring in dependence on the fluoroquinolone physicochemical properties. All fluoroquinolones accumulate within bacteria very rapidly, so that within a few minutes a steady-state intrabacterial concentration is obtained (Piddock, 1994). Fluoroquinolones are known to gain entry into phagocyticcells and remain microbiologically active inside the cells against bacterial pathogens such as *Legionella pneumophyla* (Carlier *et al*., 1990). Microscopically, the morphologic alterations produced by fluoroquinolones include decreased cell division, filamentation and cellular lysis (Foerster, 1987). Ultrastructurally, altered cell division is also evident, and bacterial cell "ghosts", i.e. remnants of the outer bacterial cell wall without internal cell components, are prominent after enrofloxacin treatment of bacterial cultures in vitro (Voight,1987). These observations may be the result of the cascade of events resulting from the inhibition of DNA gyrase leading to general bacterial cellular dysfunction, disruption of normal cellular replication and repair processes, and cell death.

1.6.2.2 Lincosamides

Lincosamides (e.g. lincomycin, clindamycin) are a class of antibiotics. Lincosamides are derived from an amino acid and a sulphur-containing octose, a synthetic monosaccharide containing eight carbon atoms in a molecule. Lincosamides prevent bacteria replicating by interfering with the synthesis of proteins. Lincosamides are characterized by a fairly broad spectrum of activity against anaerobes, like *Bacteroides species* and a limited spectrum against aerobes. Many Gram-positive cocci are inhibited but most Gram-negative bacteria, mycoplasmas and strains of *Clostridium difficile* are resistant. They bind to the 23s portion of the 50S subunit of bacterial ribosomes and cause premature dissociation of the peptidyl-tRNA from the ribosome. This site is also targeted by chloramphenicol, macrolides, quinupristin-

dalfopristin and linezolid. Due to the shared site of activity, these drugs can be antagonistic to each other and lincosamides should not be administered concurrently with erythromycin, chloramphenicol or most bactericidal agents. Lincosamides do not interfere with protein synthesis in human cells (or those of other eukaryotes) because human ribosomes are structurally different from those of bacteria. The first lincosamide to be discovered is lincomycin, isolated from *Streptomyces lincolnensis* in a soil sample from Lincoln, Nebraska. This use of lincomycin has been largely superseded by clindamycin which exhibits improved antibacterial activity.

Clindamycin also exhibits some activity against parasitic protozoa and has been used for toxoplasmosis and malaria. Lincosamides are normally used to treat Staphylococcus and Streptococcus and have proved useful in treating *Bacteroides fragilis* and some other anaerobes. They are used in the treatment of toxic shock syndrome and thought to directly block the M protein production that leads to the severe inflammatory response. Lincosamide antibiotics are one of the classes of antibiotics most associated with pseudomembranous colitis caused by *Clostridium difficile.* Target bacteria may alter the drug's binding site (similar to resistance found in macrolides and streptogramins). The resistance mechanism is methylation of the 23s binding site. If this occurs then the bacteria are resistant to both the macrolides and the lincosamides. Also, enzymatic inactivation of clindamycin has been described (rare). The lincosamides can be bacteriostatic or bactericidal depending on concentration and are more stable in hydrochloride and phosphate salts. Activity is enhanced in alkaline environments. The lincosamides, as the hydrochloride salt, are bitter to taste, so for oral formulation they are given as the palmitate esters, or formulated in capsules. Clindamycin is given intravenously as clindamycin phosphate, which is then converted into active clindamycin within the body. Lincosamides do not reach significant levels in the CSF, even in the presence of meningeal inflammation, but are well distributed in many fluids and tissues, including bone. In many species these drugs have the ability to diffuse across the placenta. The unchanged antibiotics and many metabolites are excreted in bile, urine and milk; proportions of which are determined by route of administration. Lincosamides can elevate serum alkaline phosphatase, ALT and AST on laboratory tests. Faecal levels remain high for several days after administration and microorganisms in the colon may be suppressed for up to two weeks. Kaolin-pectin preparations prevent absorption of lincosamides from the gastrointestinal tract. Use of lincosamides may result in GI disturbances. Pseudomembranous enterocolitis caused by toxigenic *Clostridium difficile* is a serious adverse reaction seen in humans. The possibility of

development of fatal colitis precludes the use of lincosamides in horses. Although no serious organ toxicities have been reported, skeletal muscle paralysis is observed with very high concentrations and additive neuromuscular effects are observed with concurrent use of anesthetic agents and skeletal muscle relaxants. Lincosamides are contraindicated in neonates due to decreased capacity to metabolize the chemical.

1.6.2.3 Macrolides

The macrolides are a group of drugs (typically antibiotics) whose activity stems from the presence of a macrolide ring, a large macrocyclic lactone ring to which one or more deoxy sugars, usually cladinose and desosamine, may be attached. The lactone rings are usually 14-, 15-, or 16-membered. Macrolides belong to the polyketide class of natural products.Antibiotic macrolides are used to treat infections caused by Gram-positive (e.g. *Streptococcus pneumoniae*) and limited Gram-negative (e.g., *Bordetella pertussis*, *Haemophilus influenzae*) bacteria, and some respiratory tract and soft-tissue infections.

The antimicrobial spectrum of macrolides is slightly wider than that of penicillin, and, therefore, macrolides are a common substitute for patients with a penicillin allergy. Beta-hemolytic streptococci, pneumococci, staphylococci, and enterococci are usually susceptible to macrolides. Unlike penicillin, macrolides have been shown to be effective against *Legionella pneumophila*, mycoplasma, mycobacteria, some rickettsia, and chlamydia. Macrolides are protein synthesis inhibitors. The mechanism of action of macrolides is inhibition of bacterial protein biosynthesis, and they are thought to do this by preventing peptidyltransferase from adding the growing peptide attached to tRNA to the next amino acid(similarly to chloramphenicol) as well as inhibiting ribosomal translation. Another potential mechanism is premature dissociation of the peptidyl-tRNA from the ribosome. Macrolide antibiotics do so by binding reversibly to the P site on the subunit 50S of the bacterial ribosome. This action is considered to be bacteriostatic. Macrolides are actively concentrated within leukocytes, and are, therefore, transported into the site of infection. The macrolide antibiotics erythromycin, clarithromycin, and roxithromycin have proven to be an effective long-term treatment for the idiopathic, Asian-prevalent lung disease diffuse panbronchiolitis (DPB). The successful results of macrolides in DPB stems from controlling symptoms through immunomodulation (adjusting the immune response) with the added benefit of low-dose requirements. With macrolide therapy in DPB, great reduction in bronchiolar inflammation and damage is achieved through suppression of not only neutrophil granulocyte proliferation but also lymphocyte activity and

obstructive secretions in airways. The antimicrobial and antibiotic effects of macrolides, however, are not believed to be involved in their beneficial effects toward treating DPB. This is evident, as the treatment dosage is much too low to fight infection, and in DPB cases with the occurrence of the macrolide-resistant bacterium *Pseudomonas aeruginosa*, macrolide therapy still produces substantial anti-inflammatory results. Apart from their antibacterial activity, these agents exhibit a broad spectrum of pharmacological effects (Bryskier *et al*., 1994), including anti-inflammatory activity in humansand animals (Tarayre *et al*., 1987; Mikasa *et al*., 1992; Agen *et al*., 1993).

Macrolides have been shown to affect several pathways of the inflammatory process, such as the migration of neutrophils, the oxidative burst in phagocytes, and the production of proinflammatory cytokines (Takeshita *et al*., 1989; Hand *et al*., 1990; Mikasa *et al*., 1992; Konno *et al*., 1994). Although the precise mechanisms of these effects are not clear, it has been suggested that the interaction between macrolides and leukocytes may be important. In fact, macrolide antibiotics are able to accumulate into polymorphonuclear leukocytes, reaching intracellular concentrations far higher than those attained in the extracellular fluids (Laufen *et al*., 1985; Hand *et al*., 1987). This ability may in turn alter the functions of phagocytes, which appear crucial for both the antibacterial defence and the inflammatory process often associated with infections. Some have suggested that the antioxidant properties, shared by several macrolides (Labro *et al*., 1989), may play a role in the anti-inflammatory activity of these agents (Plewig and Schopf, 1976; Dalziel *et al*., 1987). Although the evidence so far accumulated shows that macrolides do exert both local and systemic anti-inflammatory effects, the mechanisms underlying these actions are still unclear.

1.7 Mechanism of Platelet Aggregation

Aggregation involves platelet-to-platelet adhesion, and is necessary for effective haemostasis following the initial adhesion of platelets to the site of injury. Following adhesion, platelets are activated by a number of agonists such as adenosine diphosphate (ADP) and collagen present at the sites of vascular injury. These agonists activate platelets by binding to specific receptors on the platelet surface. Occupancy of these receptors leads to a series of downstream events that ultimately increases the intracystoplasmic concentration of calcium ions. The increase in platelet intracellular calcium occurs through release from intracellular stores and calcium influx through the plasma membrane (Varga-Szabo *et al*., 2009). Receptors coupled to G-proteins such as those to ADP, thromboxane A_2 (TXA_2) and thrombin, activate phospholipase Cβ

(PLCβ), whereas receptors acting via the non-receptor tyrosine kinase pathways such as collagen receptor GpVI preferentially activate phospholipase Cγ (PLCγ) (Varga-Szabo *et al*., 2008) activation of PLCβ or PLCγ results in the production of two second messengers: diacylglycerol (DAG) and inositol triphosphate (IP3). DAG mediates calcium influx while IP3 liberates calcium from intracellular stores. Increased platelet free calcium concentration results in a number of structural and functional changes of the platelet. Morphologically, the platelet changes dramatically from a disc to a spiny sphere (a process called shape change). The granules in the platelet are centralized and their contents are discharged into the lumen of the open canalicular system, from which they are then released to the exterior (the release reaction). The increase in Platelet calcium stimulates phospholipase A_2 activity, which liberates arachidonic acid from membrane phospholipids. Arachidonic acid is converted to an intermediate product, prostaglandin H_2 (PGH_2) by the enzyme cyclooxygenase 1 (COX-1). PGH_2 is further metabolized to $TXA_2$by thromboxane synthase (Gryglewski, 2008). TXA_2 is a potent activator of platelets. The long membrane projections brought about by shape-change reaction allow the platelets to interact with one another to form aggregates. Shape change is mediated by the platelet cytoskeleton, composed by an organized network of microtubules and actin filaments and a number of associated proteins, linked to a variety of platelet signalling molecules (Fox, 2001). Platelet shape change results in extensive reorganization of the cytoskeleton network, polymerization of actin, and myosin light chain phosphorylation (Fox, 2001; Escolar *et al*., 1986; Daniel *et al*., 1984; Jennings *et al*., 1981). These responses vary in a time- and stimulus-dependent manner. A main adhesion molecule involved in platelet aggregation is the membrane protein, GPIIb/IIIa complex. GPIIb/IIIa is an integrin receptor present at high density on platelets, both on the plasma membrane and on α-granules (Niiya *et al*., 1987). It exists as an inactive form in resting platelets.

Platelet activation by almost all agonists induces conformational changes (inside-out signalling) of GPIIb/IIIa, which becomes competent to bind soluble plasma fibrinogen. In turn, ligand binding of GPIIb/IIIa results in conformational changes directed to the cytoplasm (outside-in signalling). The precise sequence of events leading to these signalling events has not been fully elucidated (Ma *et al*., 2007; Shattil and Newman, 2004). The receptor bound fibrinogen acts as a bridge between two GPIIb/IIIa molecules on adjacent platelets (Varga-Szabo *et al*., 2008). This is the final common pathway of platelet aggregation induced by platelet chemical agonists. Although GPIIb/IIIa is the most widely studied mediator of bridging platelets to each other and stabilizing thrombi, other molecules have been proposed recently to

mediate these responses. These include junctional adhesion molecules (JAMs), SLAM (signalling lymphocyte activation molecule) family proteins and CD40 ligand (Sobocka *et al*., 2004; Nanda *et al*., 2005; Andre *et al*., 2002).

Activated platelets recruit additional platelets to the growing haemostatic plug by several feedback amplification loops: they release platelet agonists (such as ADP and serotonin stored in the α-granules) and they synthesize de novo pro-aggregatory TXA_2. Release of ADP and TXA_2 synthesis consolidates the initial haemostatic plug by promoting the participation of other platelets in the haemostatic plug formed at sites of vascular injury. Finally, platelets also play a dominant role in secondary haemostasis by providing a highly effective catalytic surface for activation of the coagulation cascade. When platelets are activated, negatively charged phospholipids move from the inner leaflet of the membrane bilayer to the outer leaflet. The transbilayer movement of anionic phospholipids is associated with blebbing and release of procoagulant vesicles rich in anionic phospholipids. Both activated platelets and the micro vesicles provide binding sites for enzymes and cofactors of the coagulation system, which then efficiently generates thrombin, itself a potent platelet agonist.

1.7.1 *In vivo* examination of platelet aggregation

Traditional approaches to monitor platelet aggregation involve exposure of platelets in suspension to a variety of stimuli *ex vivo,* a technique known as platelet aggregometry. Agonists used commonly include adenosine diphosphate (ADP), collagen, thrombin, and thromboxane A_2among others (Rand *et al*., 2003). Studies are performed on either platelet-rich plasma or whole blood and the platelets are maintained in suspension. Following exposure to an agonist, formation of platelet aggregates results in an increase in light transmission (i.e. decrease in light absorbance) through the sample. The kinetics of the responses and maximal aggregation provide quantitative assessment of platelet aggregation.

1.8 Leukocyte Mobilization

Migration of leukocytes to sites of injury or inflammation is a crucial component of both innate and adaptive immunity. To achieve this, finely co-ordinated mechanisms exist by which intravascular leukocytes are able to penetrate the vascular wall and migrate to sites of injury or infection without causing any perceptible damage to the vessels from which they emigrate. Within this scenario, leukocyte transmigration through vessel walls (predominantly post-capillary venules) is the final stage of a stepwise cascade of leukocyte responses mediated by

a series of sequential molecular interactions that initially mediate the slowing down of leukocyte rolling velocity followed by leukocyte firm adhesion to the endothelium and eventually migration through the vessel wall. Leukocyte transmigration not only acts asa means of directing the emigration of leukocytes from the vascular lumen to the extravascular tissue but may also play a critical role in regulating the phenotype of the emigrated cells such that leukocyte behaviour in the form of responsiveness to chemoattractants, directional migration and interactions with components of the extravascular tissue may be regulated. Such a mechanism could clearly contribute to the ability of the immune system to mount an optimum, appropriate and localised tissue response to a wide range of extravascular stimuli without causing any untoward damage to the vasculature. However, in conditions of uncontrolled leukocyte infiltration that may be excessive either in magnitude and/or duration, or in appropriate in its location, transmigration-induced change in phenotype of leukocytes may lead to induction or exacerbation of deleterious inflammatory responses in the host. Collectively, understanding the molecular interactions that drive and regulate the response of leukocyte transmigration could therefore be of value in the development of therapeutic strategies aimed at promoting or suppressing the host's inflammatory response, interventions that may be of potential benefit in both physiological and pathological scenarios. Venular walls are composed of endothelial cells and pericytes embedded within a tough, thin and distensible basement membrane, composed of several extracellular matrix proteins such aslaminin, collagen type IV and entactin (Erickson and Couchman, 2000). Under normal circumstances vessel walls provide a formidable barrier to cellular and non-cellular components of flowing blood, the barrier being effectively breached, however, by emigrating leukocytes and plasma proteins at sites of inflammation. Although the tight and adherens junctions provide an effective barrier to emigrating leukocytes, it is generally considered that the dominant route by which leukocytes penetrate the endothelial cell barrier is via a paracellular route i.e. through endothelial cell junctions. Leukocytes may preferentially migrate through sites where the junctional complexes are less tight, i.e. at tricellular corners as has been demonstrated by Burns et al., (1997) and/or may simply cause transient disruption of the VE-cadherin-catenin complex as they penetrate the endothelial cell junctions (Shaw *et al*, 2001). There is however at present a renewed interest in the potential ability of leukocytes to migrate through endothelial cells via a trans-cellular route, i.e. migration through the body of the endothelium, a debate that has been rekindled by the work of Feng *et al* (1998).

In this study, through detailed electron microscopic analysis of FMLP-injected guinea-pig skin sites, the authors provide convincing results indicating leukocyte migration via a transcellular route (Feng *et al*, 1998). The significance of these observations to leukocyte migration in different tissues and in response to different stimuli is at present unclear. Despite the above debate regarding the route of leukocyte emigration, there is no doubt that endothelial cell junctions do express key molecules that can support leukocyte trans- endothelial cell migration. These include PECAM-1 (CD31), CD99,ICAM-2, and members of the JAM family, molecules for which there is now convincing evidence with respect to a functional role in leukocyte transmigration from either *in vitro* and/or *in vivo* studies. Details of the precise involvement of such molecules is however currently unknown and is likely to be complex as the available data implies differential roles of endothelial cell junctional molecules in mediating different stages of leukocyte transmigration and/or transmigration of different leukocyte subtypes as well as perhaps mediating leukocytes transmigration indifferent vascular beds. Other key factors that are now known to play a significant role in regulating leukocyte migration through endothelial cells include the nature and localisation of chemokines, i.e. lumenal or ablumenal that may determine the ability of leukocytes to migrate on and through the endothelium. In addition, there is now clear in vitro evidence for a requirement for physiological shear stress in promoting/accelerating trans-endothelial migration of leukocytes across stimulated cultured endothelial cells (Cinamon *et al*., 2001; Kitayama *et al*., 2000).Leukocyte transmigration is studied in vivo using a number of models. The most common approach is to quantify the migration of leukocytes into a body cavity such as the pleural or peritoneal cavity following the local instillation of a non-specific pro-inflammatory stimulus such as thioglycollate or glycogen, or as commonly used by our group, a defined inflammatory mediator such as IL-1β or IL-4 (Dangerfield *et al*, 2002; Larbi *et al*, 2000; Larbi *et al*, 2003). Of course, leukocyte infiltration can be induced in any organ (e.g. skin, lung, heart) and tissues analysed ex-vivo by standard histological and/or electron microscopic techniques.

1.9 Aim of the study

The aim of this study was to investigate the anti-inflammatory and analgesic activities of certain antibiotics (Ciprofloxacin, Lincomycin and Erythromycin) *in vivo* and *in vitro*.

1.10 Specific Objectives

1. To assay for the effect of the three antibiotics on agar-induced paw oedema in Wistar rats.

2. To assay for the analgesic activities of the three antibiotics on thermal-induced pain in Wistar rats.
3. To assay for the effect of the three antibiotics on agar-induced leucocyte mobilization to the peritoneal cavity.
4. To assay for the effect of the three antibiotics on phospholipase A_2 activity.
5. To assay for the effect of the three antibiotics on platelet aggregation.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment and Apparatus

Syringes: 10ml, 5ml, 2ml and 1ml disposable hypodermic syringes manufactured by DANA City were used for various purposes.

Feeding Cannula: This was used to administer the drugs to the animals orally. They were bent and blunted to prevent injury on the animals.

Capillary tubes: They were used for obtaining blood from the animals

Sample bottles were used for storing the prepared drug solutions.

Conical flasks of 250ml volume were used for the submerged culture of *Aspergillus niger.*

JENWAY Spectrophotometer (UK) was used to measure the absorbance of various samples.

Centrifuge: used for sedimentation and separation of samples.

Hot Plate: This was used for inducing pain on the rats in the Analgesic activity test.

Thermometer: This was used for checking the temperature during the Analgesic activity test

2.1.2 Chemicals and Reagents

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

3% agar suspension: A 3% (w/v) agar suspension was prepared by dissolving 3g of agar in 100ml of distilled water.

5% EDTA Solution: A 5% solution of EDTA was prepared by dissolving 5g of EDTA powder in 100ml of distilled water.

Phosphate buffered saline: This solution was prepared by dissolving 3.90g of $NaH_2PO_4.2H_2O$ and 4.45g of $Na_2HPO_4.2H_2O$ in 250ml of distilled water. 40.5ml aliquot of $Na_2HPO_4.2H_2O$ 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.

2mM $CaCl_2$: This was prepared by dissolving 0.022g of $CaCl_2$ in 100ml of distilled water and used in the Phospholipase A_2 assay.

1.47% $CaCl_2$: This was prepared by dissolving 1.47g of $CaCl_2$ in 100ml of distilled water and used for the anti-platelet aggregatory activity.

SDA broth: This was prepared by dissolving 15g of Sabouraud dextrose agar in 1000ml of distilled water. It was used as the media for the submerged culture of *Aspergillus niger* for the preparation of the crude Phospholipase A_2 enzyme.

Nutrient agar: Prepared by dissolving 28.0g of nutrient agar in 1000ml of distilled water. The mixture was homogenized in a water bath at 100^oC for 10 minutes; 5ml was dispensed into petridishes and autoclaved at 121^oC for 15minutes. It was used for the first culture of *Aspergillus niger*.

2.1.3 Drugs

Ciprofloxacin (Ciprotab) used were ciprofloxacin hydrochloride U.S.P. (ciprofloxacin hydrochloride, 500mg) soft gelatin coated tablets.They were manufactured by Geltec Pvt. Ltd., Karnataka, India. They served as a test drug.

Lincomycin used was Lincomycin 500mg capsules. They were manufactured by Mekophar (Ho Chi Minh City, Vietnam) and used as a test drug.

Erythromycin used were erythromycin stearate (erythromycin base, 500mg) film coated tablets. They were manufactured by Mekophar (Ho Chi Minh City, Vietnam). They served as a test drug.

Prednisolone (Predicure) used were prednisolone 5mg round tablets. They were manufactured by Unicure Pharmaceuticals Ltd (Lagos, Nigeria). They served as the reference drug for the phospholipase A_2 Activity assay.

Indomethacin used were indomethacin 25mg capsules. They as a reference drug for paw oedema, leukocyte mobilization, and anti-platelet aggregation assays.

Acetylsalicylic acid (Aspirin) used were 300mg tablets. They were used as the reference drug for the analgesic activity test.

2.1.4 Experimental Animals

Three groups of female Wistar albino rats weighing between 63g and 160 g were used for this study. The first group comprised rats weighing 63g to 82g while the other two groups comprised rats weighing 130g to 160g. Animals were housed in aluminium cages. The light cycle was controlled (12hr day/night cycle), and the room temperature was approximately 28±2ºC. Before the experiments, the animals were housed in these conditions for 4 days to become acclimatized. They were fed TOP finisher pelletized feeds and allowed free access to water. The blood samples used for the platelet aggregation tests were collected from Wistar rats. The blood sample for the phospholipase activity was collected from apparently healthy individuals. They were free from drug treatments for at least 2 weeks before sample collection. The fungal organisms used were strains of *Aspergillus niger* which were cultured in the Department of Microbiology, University of Nigeria, Nsukka.

2.2 Methods / Experimental Design

2.2.1 Effect of Antibiotic on Agar Induced Paw Oedema

The rat paw oedema method of Winter *et al*. (1962) was used for the test. Increase in the right hind limb paw volume induced by the sub-plantar injection of 3% w/v agar solution was used as a measure of acute inflammation. Thirty three adult female Wistar rats were divided into 11 groups of 3 rats each. The animals were allowed to acclimatise for 6 days before the experiment. The animals were then fasted and deprived of water for 18h before the experiment to ensure uniform hydration and to minimize variability in oedematous response. After deprivation, the right hind paw volume of the rats were measured at time zero (t=0) with a Vernier calliper. Three groups of 3 rats each were treated with ciprofloxacin (Cipro 10mg/kg, 20mg/kg and 40mg/kg) orally. One hour after receiving the drug, each animal received a subcutaneous injection of the inflammatory agent in the right hind paw. The oedema was measured immediately prior to the injection of the inflammatory agent and 30min, 1h, 2h, 3h, 4h and 5h after. The paw volume was determined in millilitres as the difference between the final and initial volumes. The same procedure was replicated with the remaining antibiotics: lincomycin and erythromycin. One group of three rats each was treated with the reference drug, indomethacin 5mg/kg orally. One hour after receiving the drug, each animal received a subcutaneous injection of the inflammatory agent in the right hind paw. The oedema was measured as stated above.

One group of 3 rats each was treated with the vehicle, normal saline. One hour after receiving the vehicle, each animal received a subcutaneous injection of the inflammatory agent in the right hind paw. The oedema was measured as stated above. This served as the control.

% Inflammation was given as $100\ [1 - \frac{(a-x)}{(b-y)}]$

Where a = mean paw volume of treated rats after inflammation.

x = mean paw volume of treated rats before inflammation.

b = mean paw volume of control rats after inflammation.

y = mean paw volume of control rats before inflammation.

2.2.2 Analgesic Activity Test

Analgesic activity was tested in Wistar rats using the hot plate method of Janssen and Jagneau (1957). Thirty three female Wistar rats were divided into 11 groups (n=3 per group). The first 3 groups received ciprofloxacin (Cipro 50mg/kg, 100mg/kg, and 200mg/kg) orally. The next 3 groups received lincomycin (Linco 50mg/kg, 100mg/kg, and 200mg/kg) orally. The next 3 groups received erythromycin (Erythro 50mg/kg, 100mg/kg and 200mg/kg) orally. The remaining 2 groups received acetylsalicylic acid (Asp 100mg/kg) and normal saline (5ml/kg) respectively. A 500ml glass beaker was placed on the hot plate and the temperature regulated to 50 ± 2 ^{o}C. The animals were lowered onto the surface of the beaker and the time for the animal to raise or lick the fore limb was noted as the reaction time. Cut off time in the absence of a response was 90sec to prevent the animals from being burnt. (Sharma *et al*., 1982).

2.2.3 Effect of Antibiotic on Leukocyte Mobilization

The effect of the test antibiotics on *in vivo* leukocyte mobilization induced by an inflammatory stimulus was investigated using the method described by Ribeiro *et al* (1991). Thirty three adult female Wistar albino rats were divided into 11 groups of 3 animals each. The groupings are a replica of groupings under the paw oedema. The remaining 2 groups received indomethacin (Indo 5mg/kg) and normal saline respectively. 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 (PBS).

The peritoneal fluid was recovered. Total leukocyte count (TLC) and differential leukocyte count (DLC) were performed on the peritoneal fluid.

2.2.4 Effect of Antibiotics on Phospholipase A_2 Activity

Determination of the effect of the antibiotics on phospholipase A2 activity was by the method of Vane (1971). Nutrient agar plates were prepared by dissolving 28.0g of nutrient agar in 1000ml of distilled water. The nutrient agar solution was homogenized in a water bath, 100^{o}C, for 10min and dispensed into sterilized Petridishes. The petridishes containing the agar were autoclaved at 121^{o}C for 15minutes. A sample of *Aspergillus niger* was then inoculated on the plates and left to grow for 3 days. The nutrient broth was prepared by dissolving 15g of Sabouraud dextrose agar in 1000ml of distilled water, homogenized in a water-bath for 10min and dispensed into 250ml conical flasks. The conical flasks were sealed with cotton wool and foil paper. The broth was then autoclaved at 121^{o}C for 15 minutes. The broth was allowed to cool to room temperature and then the organisms in the petridishes were aseptically inoculated into the broth and incubated for 72 h at room temperature. The culture was transferred into test tubes containing 3ml phosphate buffered saline and centrifuged at 3000g for 10 min. The fungal cells settled at the bottom of the test tube while the supernatant was used as the crude enzyme preparation. Various blood samples (5ml) were drawn out from apparently healthy individuals and dispensed into plastic tubes containing 0.01ml of 1% EDTA as an anticoagulant. They were centrifuged at 3000g for 10 minutes and the supernatant (plasma) discarded. The red cells were re-suspended in a volume of normal saline equal to that of the plasma, centrifuged at 3000g for 10 minutes and the supernatant discarded. The red cells were re-suspended in a volume of normal saline equal to the discarded supernatant. This solution of red cells was reconstituted as a 40% (v/v) suspension with phosphate buffered saline and this served as the substrate for phospholipase. $CaCl_2$ (2mM) (0.2ml), human red blood cell (HRBC) (0.2ml), 0.2ml of the crude enzyme preparation and varying concentrations of normal saline and the test drug or reference drug were incubated in test-tubes for 1hr. The control contained the human red blood cell suspension, $CaCl_2$ and free enzyme. The blanks were treated with 0.2ml of boiled enzyme separately. The incubation reaction mixtures were centrifuged at a speed of 3000g for 10 minutes. Samples of the supernatant (1.5ml) were diluted with 10ml of normal saline and the absorbance of the solutions read at 418nm. Prednisolone, a known inhibitor of phospholipase A2, was used as the reference drug (Morimoto *et al*., 1979).

2.2.5 Assay of Anti-Platelet Aggregatory Activity

The assay used is a modification of the method of Born and Cross (1963). Various blood samples were taken from 3 adult Wistar albino rats with the aid of a capillary tube. Fresh blood (10ml each) were drawn into plastic tubes containing 1% EDTA droplets as an anticoagulant. The tubes were centrifuged at 3000rpm for 10minutes. The plasma supernatant was collected, diluted twice with normal saline and used as the platelet rich plasma (PRP). The test drugs were dissolved 1mg/ml in distilled water. Platelet rich plasma (0.2ml), 0.4ml of 1.47% $CaCl_2$ and varying concentrations of normal saline and the drug were incubated at room temperature for 2min. Indomethacin was used as the reference drug. $CaCl_2$ was used to start the reaction. The absorbance of the solutions were measured at 520nm. Changes in the absorbance were taken at zero seconds, 30sec, 60sec, 90sec and 120sec after adding $CaCl_2$.

CHAPTER THREE

RESULTS

3.1 Effect of Ciprofloxacin on agar-induced paw oedema of rats:

Table 1 shows the effect of ciprofloxacin on rat paw oedema induced in rats with 3% agar. It shows that agar induced oedema in the rat paw was sustained over a period of 5 hours. Groups treated with 10mg/kg, 20mg/kg and 40mg/kg of ciprofloxacin and 5mg/kg of indomethacin showed a significant reduction in paw oedema ($p<0.05$) when compared with the control. The paw size of animals treated with increasing doses of ciprofloxacin and indomethacin significantly decreased with time. The paw size of the control animals did not show any significant difference over the 5 hours. The percentage oedema inhibition for rats treated with ciprofloxacin 10mg/kg after 30min, 1h, 2h, 3h, 4h, and 5h were 33.33%, 48.00%, 46.43%, 57.69%, 69.23% and 88.46% respectively. The percentage oedema inhibition for rats treated with ciprofloxacin 20mg/kg after 30min, 1h, 2h, 3h, 4h and 5h were 37.03%, 48.00%, 60.71%, 69.23%, 84.62% and 96.15% respectively. The percentage oedema inhibition for rats treated with ciprofloxacin 40mg/kg after 30min, 1h, 2h, 3h, 4h and 5h were 22.22%, 32.00%, 46.43%, 50.00%, 69.23% and 53.85% respectively. The percentage oedema inhibition for rats treated with indomethacin 5mg/kg after 30min, 1h, 2hr, 3hr, 4hr, and 5hr was 18.52%, 24.00%, 28.57%, 46.15%, 46.20% and 61.54% respectively. The percentage oedema inhibition of the three (3) doses of ciprofloxacin was higher than that of indomethacin. The effect of the drug was dose dependent but showed a deviation at the highest dose of the drug (40mg/kg). The percentage inhibition at this dose was lower over the time period when compared with the values obtained for 10mg/kg and 20mg/kg.

Table 1: Inhibition by Ciprofloxacin of agar-induced rat paw oedema.

GROUP	Mean Oedema (cm) and Duration					
	30 Minutes	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
Cipro 10mg/kg	0.19 ± 0.03^{abB}	0.14 ± 0.06^{aAB}	0.15 ± 0.10^{abB}	0.11 ± 0.04^{abAB}	0.08 ± 0.06^{abcAB}	0.03 ± 0.04^{abA}
	# 33.33%	# 48.00%	# 46.43%	# 57.69%	# 69.23%	# 88.46%
Cipro 20mg/kg	0.17 ± 0.06^{aD}	0.13 ± 0.03^{aCD}	0.11 ± 0.04^{aBCD}	0.09 ± 0.02^{aBC}	0.05 ± 0.04^{aAB}	0.02 ± 0.02^{aA}
	# 37.03%	# 48.00%	# 60.71%	# 69.23%	# 84.62%	# 96.15%
Cipro 40mg/kg	0.21 ± 0.08^{abB}	0.17 ± 0.06^{abAB}	0.15 ± 0.03^{abAB}	0.13 ± 0.03^{abAB}	0.08 ± 0.06^{abcA}	0.12 ± 0.02^{cAB}
	# 22.22%	# 32.00%	# 46.43%	# 50.00%	# 69.23%	# 53.85%
Indo 5mg/kg	0.21 ± 0.03^{abB}	0.18 ± 0.04^{abAB}	0.20 ± 0.05^{abAB}	0.14 ± 0.08^{abAB}	0.14 ± 0.06^{bcAB}	0.10 ± 0.04^{bcA}
	# 18.52%	# 24.00%	# 28.57%	# 46.15%	# 46.20%	# 61.54%
Normal Saline	0.27 ± 0.05^{bA}	0.24 ± 0.04^{bA}	0.28 ± 0.05^{cA}	0.26 ± 0.04^{cA}	0.26 ± 0.01^{dA}	0.26 ± 0.08^{dA}

n = 3
Results expressed as Mean ± SD
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column.
Mean values having different uppercase letters as superscripts are considered significant ($p < 0.05$) across the row.

represents percentage inhibition of paw oedema calculated relative to control.

3.2. Effect of Lincomycin on agar-induced paw oedema of rats:

Table 2 indicates the effect of lincomycin on the paw oedema induced in rats with 3% agar. The result showed that agar induced oedema in the rat paw was sustained over a period of 5 hours. Groups treated with 10mg/kg, 20mg/kg and 40mg/kg of lincomycin and 5mg/kg of indomethacin showed a significant reduction in paw oedema ($p<0.05$) when compared with the control. The paw size of animals treated with 20mg/kg, 40mg/kg of lincomycin and indomethacin significantly ($p<0.05$) decreased with time. Lincomycin 10mg/kg showed no significant decrease over time. There was no significant decrease in the paw size of control animals across the 5 h period. The percentage oedema inhibition values for rats treated with lincomycin 10mg/kg after 30min, 1h, 2h, 3h, 4h, and 5h were 51.85%, 52.00%, 67.86%, 53.85%, 76.92% and 65.38% respectively. The percentage oedema inhibition values for rats treated with lincomycin 20mg/kg after 30min, 1h, 2h, 3h, 4h and 5h were 29.62%, 44.00%, 57.14%, 57.69%, 73.08% and 84.62% respectively. The percentage oedema inhibition values for rats treated with lincomycin 40mg/kg after 30min, 1h, 2h, 3h, 4h and 5h were 33.33%, 44.00%, 53.57%, 69.23%, 68.00% and 92.31% respectively. The percentage oedema inhibition values for rats treated with indomethacin 5mg/kg after 30min, 1hr, 2hr, 3hr, 4hr, and 5hr were 18.52%, 24.00%, 28.57%, 46.15%, 46.20% and 61.54% respectively. The effect of the drug was dose dependent. The percentage oedema inhibition values of each of the three (3) doses of lincomycin was higher than that of indomethacin.

Table 2: Inhibition by Lincomycin of agar-induced rat paw oedema

GROUP	Mean Oedema (cm) and Duration					
	30 Minutes	**1 Hour**	**2 Hours**	**3 Hours**	**4 Hours**	**5 Hours**
Linco 10mg/kg	0.13 ± 0.02^{aA}	0.12 ± 0.04^{aA}	0.09 ± 0.08^{aA}	0.12 ± 0.36^{abA}	0.06 ± 0.07^{abA}	0.09 ± 0.07^{abcA}
	# 51.85%	# 52.00%	# 67.86%	# 53.85%	# 76.92%	# 65.38%
Linco 20mg/kg	0.18 ± 0.06^{abD}	0.14 ± 0.05^{aCD}	0.11 ± 0.02^{abBC}	0.11 ± 0.02^{abBC}	0.07 ± 0.04^{abAB}	0.04 ± 0.00^{abA}
	# 29.62%	# 44.00%	# 57.14%	# 57.69%	# 73.08%	# 84.62%
Linco 40mg/kg	0.19 ± 0.05^{abD}	0.15 ± 0.01^{aCD}	0.13 ± 0.02^{abBC}	0.08 ± 0.04^{aB}	0.08 ± 0.02^{abcB}	0.02 ± 0.02^{aA}
	# 33.33%	# 44.00%	# 53.57%	# 69.23%	# 68.00%	# 92.31%
Indo 5mg/kg	0.21 ± 0.03^{abB}	0.18 ± 0.04^{abBC}	0.20 ± 0.05^{bdBC}	0.14 ± 0.08^{abBC}	0.14 ± 0.06^{bcBC}	0.10 ± 0.04^{bcA}
	# 18.52%	# 24.00%	# 28.57%	# 46.15%	# 46.20%	# 61.54%
Normal Saline	0.27 ± 0.05^{bA}	0.24 ± 0.04^{bA}	0.28 ± 0.05^{dA}	0.26 ± 0.04^{cA}	0.26 ± 0.01^{dA}	0.26 ± 0.08^{dA}

n = 3
Results expressed as Mean ± SD
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column.
Mean values having different uppercase letters as superscripts are considered significant ($p < 0.05$) across the row.

represents percentage inhibition of paw oedema calculated relative to control.

3.3. Effect of Erythromycin on agar-induced paw oedema of rats:

Table 3 shows the effect of erythromycin on the paw oedema induced in rats with 3% agar. The result showed that agar induced oedema in the rat paw which was sustained over a period of 5 hours. Groups treated with 10mg/kg, 20mg/kg of erythromycin and 5mg/kg of indomethacin showed a significant reduction in paw oedema ($p<0.05$) when compared with the control at every time interval. The paw size after 30 minutes was non-significantly ($p>0.05$) higher than the paw size after 5 hours in the animals treated with the increasing doses of lincomycin unlike the paw sizes of indomethacin-treated rats which showed a significant ($p<0.05$) decrease when compared with those of the control at 3hrs, 4hrs and 5hrs. The paw size of the control animals remained almost the same over 5 hours. The percentage oedema inhibition values for rats treated with erythromycin 10mg/kg after 30min, 1h, 2hr, 3hr, 4hr, and 5hr was 29.62%, 28.00%, 35.71%, 38.46%, 34.62% and 50.00% respectively. The percentage oedema inhibition values for rats treated with erythromycin 20mg/kg after 30min, 1hr, 2hr, 3hr, 4hr and 5hr was 44.44%, 32.00%, 50.00%, 38.46%, 53.85% and 61.54% respectively. The percentage oedema inhibition values for rats treated with erythromycin 40mg/kg after 30min, 1hr, 2hr, 3hr, 4hr and 5hr was 25.93%, 32.00%, 14.28%, 30.77%, 50.00% and 42.31% respectively. The percentage oedema inhibition values for rats treated with indomethacin 5mg/kg after 30min, 1hr, 2hr, 3hr, 4hr, and 5hr was 18.52%, 24.00%, 28.57%, 46.15%, 46.20% and 61.54% respectively. The percentage oedema inhibition of the three (3) doses of ciprofloxacin was higher than that of indomethacin. The effect of the drug was dose dependent but showed a deviation at the highest dose of the drug (40mg/kg). The percentage inhibition at this dose was lower over the time period when compared with the values obtained for 10mg/kg and 20mg/kg.

Table 3: Inhibition byErythromycin of agar-induced rat paw oedema

GROUP	Mean Oedema (cm) and Duration					
	30 Minutes	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
Erythro 10mg/kg	0.19 ± 0.05^{abA}	0.18 ± 0.06^{bA}	0.17 ± 0.04^{aA}	0.16 ± 0.03^{bA}	0.17 ± 0.05^{aA}	0.13 ± 0.04^{aA}
	# 29.62%	# 28.00%	# 35.71%	# 38.46%	# 34.62%	# 50.00%
Erythro 20mg/kg	0.16 ± 0.02^{aA}	0.17 ± 0.02^{bA}	0.15 ± 0.03^{aA}	0.16 ± 0.06^{bA}	0.12 ± 0.05^{aA}	0.10 ± 0.04^{aA}
	# 44.44%	# 32.00%	# 50.00%	# 38.46%	# 53.85%	# 61.54%
Erythro 40mg/kg	0.20 ± 0.06^{abAB}	0.16 ± 0.03^{bAB}	0.23 ± 0.04^{abB}	0.17 ± 0.02^{bAB}	0.13 ± 0.02^{aA}	0.14 ± 0.04^{aA}
	# 25.93%	# 32.00%	# 14.28%	# 30.77%	# 50.00%	# 42.31%
Indo 5mg/kg	0.21 ± 0.03^{abB}	0.18 ± 0.04^{bAB}	0.20 ± 0.05^{abAB}	0.14 ± 0.08^{bAB}	0.14 ± 0.06^{aAB}	0.10 ± 0.04^{aA}
	# 18.52%	# 24.00%	# 28.57%	# 46.15%	# 46.20%	# 61.54%
Normal Saline	0.27 ± 0.05^{bA}	0.24 ± 0.04^{bA}	0.28 ± 0.05^{bA}	0.26 ± 0.04^{cA}	0.26 ± 0.01^{bA}	0.26 ± 0.08^{bA}

n = 3
Results expressed as Mean ± SD
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column.
Mean values having different uppercase letters as superscripts are considered significant ($p < 0.05$) across the row.

represents percentage inhibition of paw oedema calculated relative to control.

3.4 Effect of Ciprofloxacin on thermal-induced pain in rats

Table 4 shows the effect of the antibiotic, ciprofloxacin, on thermal-induced pain in rats. The hot glass surface on which the rats were placed caused pain on the rat paw. At 15 min, Ciprofloxacin 50mg/kg, 100mg/kg and 200mg/kg had reaction times of 4.220sec, 4.363sec and 4.713sec respectively. These reaction times were all higher than the reaction time of the control animals which was 3.890sec but they were lower than that of aspirin-induced animals which had a reaction time of 4.920sec. The reaction time of the aspirin treated rats was significantly higher than that of rats treated with ciprofloxacin 50mg/kg and 100mg/kg as well as that of the control samples. At 60 min, ciprofloxacin 50mg/kg, 100mg/kg and 200mg/kg had reaction times of 6.017sec, 6.467sec and 6.927sec respectively. These reaction times were all significantly ($p<0.05$) higher than the reaction time of the control animals which was 3.950sec but they were all lower than the reaction time of aspirin-treated animals which was 8.870sec. The reaction time of the aspirin-treated rats was significantly higher than the reaction time for rats treated with all doses of ciprofloxacin as well as the control. The effect of ciprofloxacin was dose dependent. All doses (50mg/kg, 100mg/kg and 200mg/kg) of ciprofloxacin also significantly ($p<0.05$) prolonged the reaction latency at 60 minutes.

Table 4: Analgesic activity of Ciprofloxacin

GROUP	Duration(minutes) and Reaction time(seconds)	
	15 Minutes	**60 Minutes**
Cipro 50mg/kg	4.220 ± 0.036^{abA}	6.017 ± 0.101^{bB}
Cipro 100mg/kg	4.363 ± 0.124^{bA}	6.467 ± 0.076^{cB}
Cipro 200mg/kg	4.713 ± 0.156^{cA}	6.927 ± 0.085^{dB}
Aspirin 50mg/kg	4.920 ± 0.135^{cA}	8.870 ± 0.221^{eB}
Control	3.890 ± 0.192^{aA}	3.950 ± 0.209^{aA}

n = 3
Results expressed as Mean ± SD
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column.
Mean values having different uppercase letters as superscripts are considered significant ($p < 0.05$) across row.

3.5 Effect of Lincomycin on thermal-induced pain in rats

Table 5 shows the effect of the antibiotic, lincomycin, on thermal-induced pain in rats. The hot glass surface on which the rats were placed caused pain on the rat paw. At 15 min, lincomycin 50mg/kg, 100mg/kg and 200mg/kg had reaction times of 3.957sec, 3.983sec and 4.050sec respectively. These reaction times were all higher than the reaction time of the control animals which was 3.890sec but they were lower than that of aspirin-induced animals which had a reaction time of 4.920sec. The reaction time of the aspirin treated rats was significantly higher than that of rats treated with lincomycin 50mg/kg, 100mg/kg and 200mg/kg as well as the control samples. At 60 min, lincomycin 50mg/kg, 100mg/kg and 200mg/kg had reaction times of 3.970sec, 4.003sec and 3.860sec respectively. These reaction times were all lower than the reaction time of aspirin-treated animals which was 8.870sec. The reaction time of the aspirin-treated rats was significantly higher than the reaction time for rats treated with all doses of lincomycin as well as the control. The effect of lincomycin was dose dependent at 15 min. There was also no significant difference between the reaction latency of rats treated with the three doses of lincomycin at 15 minutes and 60 minutes post treatment.

Table 5: Analgesic activity of Lincomycin

GROUP	Duration(minutes) and Reaction time(seconds)	
	15 Minutes	**60 Minutes**
Linco 50mg/kg	3.957 ± 0.087^{aA}	3.970 ± 0.171^{aA}
Linco 100mg/kg	3.983 ± 0.191^{aA}	4.003 ± 0.205^{aA}
Linco 200mg/kg	4.050 ± 0.056^{aA}	3.860 ± 0.219^{aA}
Aspirin 50mg/kg	4.920 ± 0.135^{bA}	8.870 ± 0.221^{bB}
Control	3.890 ± 0.192^{aA}	3.950 ± 0.209^{aA}

n = 3
Results expressed as Mean ± SD
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column.
Mean values having different uppercase letters as superscripts are considered significant ($p < 0.05$) across row.

3.6 Effect of Erythromycin on thermal-induced pain in rats

Table 6 shows the effect of the antibiotic, erythromycin, on thermal-induced pain in rats. The hot glass surface on which the rats were placed caused pain on the rat paw. At 15 min, erythromycin 50mg/kg, 100mg/kg and 200mg/kg had reaction times of 3.560sec, 3.898sec and 3.783sec respectively. These reaction times were lower than aspirin-induced animals which had a reaction time of 4.920sec. The reaction time of the aspirin-treated rats was significantly higher than that of rats treated with erythromycin 50mg/kg, 100mg/kg and 200mg/kg as well as the control group (3.890sec). At 60 min, erythromycin 50mg/kg, 100mg/kg and 200mg/kg had reaction times of 3.453sec, 3.953sec and 3.803sec respectively. These reaction times were all lower than the reaction time of aspirin-treated animals which was 8.870sec. The reaction time of the aspirin-treated rats was significantly higher than the reaction time for rats treated with all doses of erythromycin as well as the control. The effect of erythromycin was not dose dependent. There was also no significant difference between the reaction latency of rats treated with the three doses of lincomycin at 15 minutes and 60 minutes post treatment.

Table 6: Analgesic activity of Erythromycin

GROUP	Duration(minutes) and Reaction time(seconds)	
	15 Minutes	**60 Minutes**
Erythro 50mg/kg	3.560 ± 0.450^{aA}	3.453 ± 0.497^{aA}
Erythro 100mg/kg	3.898 ± 0.067^{aA}	3.953 ± 0.045^{bA}
Erythro 200mg/kg	3.783 ± 0.154^{aA}	3.803 ± 0.199^{abA}
Aspirin 50mg/kg	4.920 ± 0.135^{bA}	8.870 ± 0.221^{cB}
Control	3.890 ± 0.192^{aA}	3.950 ± 0.209^{bA}

n = 3
Results expressed as Mean ± SD
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column.
Mean values having different uppercase letters as superscripts are considered significant ($p < 0.05$) across row.

3.7 Effect of Ciprofloxacin on agar-induced leukocyte mobilization

Table 7 shows the effect of ciprofloxacin on agar-induced leukocyte migration in rats. There was a decrease in agar-induced mobilization of leukocytes upon treatment with ciprofloxacin. This decrease was dose dependent with Ciprofloxacin 10mg/kg, 20mg/kg and 40mg/kg having total leucocyte counts of 2566.67, 2366.67 and 2000.00 respectively. Neutrophils were the most mobilized leucocytes. Basophils were barely mobilized. Indomethacin decreased leukocyte mobilization into the cavity.

Table 7: Effect of Ciprofloxacin on agar-induced leukocyte mobilization

GROUP	TLC	Differential Cell Count (%)				
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Cipro 10mg/kg	2566.67±57.74	59.33±10.07	36.67±10.50	3.00±1.00	2.00±1.00	0.00±0.00
Cipro 20mg/kg	2366.67±351.3	62.00±2.00	32.00±3.61	3.67±1.53	2.33±1.53	0.00±0.00
Cipro 40mg/kg	2000.00±100.0	59.00±4.58	36.33±7.02	2.33±2.31	2.33±0.58	0.00±0.00
Indo 5mg/kg	1866.67±152.75	58.67±6.11	37.67±7.02	2.00±1.00	1.67±0.58	0.00±0.00
Control	3666.67±208.17	63.67±4.51	31.67±6.02	3.00±1.00	2.00±0.00	0.00±0.00

n = 3
Results expressed as Mean ± SD

3.8 Effect of Lincomycin on agar-induced leukocyte mobilization

Table 8 shows the effect of lincomycin on agar-induced leukocyte migration in rats. There was a decrease in agar-induced mobilization of leukocytes upon treatment with lincomycin. This decrease was dose dependent with lincomycin 10mg/kg, 20mg/kg and 40mg/kg having total leucocyte counts of 2633.33, 2266.67 and 1900.00 respectively. Neutrophils were the most mobilized leucocytes. Basophils were barely mobilized. The total cell count was lower in indomethacin-treated group when compared to the values obtained for all doses of lincomycin-treated rats. The difference between the value obtained for indomethacin-treated group and that of the rats treated with the highest dose of lincomycin (the drug) was not significant ($p>0.05$).

Table 8: Effect of lincomycin on agar-induced leukocyte mobilization

GROUP	TLC	Differential Cell Count (%)				
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Linco 10mg/kg	2633.33±152.8	58.67±9.02	37.33±9.02	3.00±1.00	1.67±0.58	0.00±0.00
Linco 20mg/kg	2266.67±115.47	57.33±6.11	38.33±5.69	2.67±0.58	1.67±0.58	0.00±0.00
Linco 40mg/kg	1900.00±173.20	59.67±2.52	37.67±2.52	1.67±0.58	1.00±1.00	0.00±0.00
Indo 5mg/kg	1866.67±152.75	58.67±6.11	37.67±7.02	2.00±1.00	1.67±0.58	0.00±0.00
Control	3666.67±208.17	63.67±4.51	31.67±6.02	3.00±1.00	2.00±0.00	0.00±0.00

n = 3
Results expressed as Mean ± SD.

3.9 Effect of Erythromycin on agar-induced leukocyte mobilization

Table 9 shows the effect of erythromycin on agar-induced leukocyte migration in rats. There was a decrease in agar-induced mobilization of leukocytes upon treatment with ciprofloxacin. This decrease was not dose dependent but the highest concentration of the antibiotic had the least total leucocyte count. Erythromycin 10mg/kg, 20mg/kg and 40mg/kg had total leucocyte counts of 2333.33, 2733.33 and 1866.67 respectively. Neutrophils were the most mobilized leucocytes. Basophils were barely mobilized. The total cell count was lower in the group treated with indomethacin when compared to the value obtained for the groups treated with 10mg/kg and 20mg/kg erythromycin. There was no difference between the value obtained for indomethacin-treated group and that obtained for the highest dose of the drug.

Table 9: Effect of Erythromycin on agar-induced leukocyte mobilization

GROUP	TLC	Differential Cell Count (%)				
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Erythro 10mg/kg	2333.33±142.91	54.67±7.02	41.33±7.02	2.33±0.58	1.67±0.58	0.00±0.00
Erythro 20mg/kg	2733.33±208.17	57.33±1.53	37.67±1.53	3.33±0.58	1.67±0.58	0.00±0.00
Erythro 40mg/kg	1866.67±208.17	54.00±2.00	42.67±3.21	2.00±1.00	1.33±0.58	0.00±0.00
Indo 5mg/kg	1866.67±152.75	58.67±6.11	37.67±7.02	2.00±1.00	1.67±0.58	0.00±0.00
Control	3666.67±208.17	63.67±4.51	31.67±6.02	3.00±1.00	2.00±0.00	0.00±0.00

n = 3
Results expressed as Mean ± SD

3.10 Effect of the various antibiotics on agar-induced leukocyte mobilization

Table 10 is a comparative table showing the effects of the various antibiotics on leucocyte mobilization and how they compare to one another. All the antibiotics significantly reduced leucocyte mobilization into the affected tissue. They all had their maximum inhibitory effects at the highest dose (40mg/kg) and these values compared well with those obtained for indomethacin-treated groups. Erythromycin (40mg/kg) showed the highest inhibitory effect on leucocyte migration. The control had the highest total leucocyte count (3666.67) and the highest percentage of neutrophils that migrated (63.67%).

Table 10: Effect of the various antibiotics on agar-induced leukocyte mobilization

GROUP	TLC	Differential Cell Count (%)				
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Cipro 10mg/kg	2566.67±57.74	59.33±10.07	36.67±10.50	3.00±1.00	2.00±1.00	0.00±0.00
Cipro 20mg/kg	2366.67±351.3	62.00±2.00	32.00±3.61	3.67±1.53	2.33±1.53	0.00±0.00
Cipro 40mg/kg	2000.00±100.0	59.00±4.58	36.33±7.02	2.33±2.31	2.33±0.58	0.00±0.00
Linco 10mg/kg	2633.33±152.8	58.67±9.02	37.33±9.02	3.00±1.00	1.67±0.58	0.00±0.00
Linco 20mg/kg	2266.67±115.47	57.33±6.11	38.33±5.69	2.67±0.58	1.67±0.58	0.00±0.00
Linco 40mg/kg	1900.00±173.20	59.67±2.52	37.67±2.52	1.67±0.58	1.00±1.00	0.00±0.00
Erythro 10mg/kg	2333.33±142.91	54.67±7.02	41.33±7.02	2.33±0.58	1.67±0.58	0.00±0.00
Erythro 20mg/kg	2733.33±208.17	57.33±1.53	37.67±1.53	3.33±0.58	1.67±0.58	0.00±0.00
Erythro 40mg/kg	1866.67±208.17	54.00±2.00	42.67±3.21	2.00±1.00	1.33±0.58	0.00±0.00
Indo 5mg/kg	1866.67±152.75	58.67±6.11	37.67±7.02	2.00±1.00	1.67±0.58	0.00±0.00
Control	3666.67±208.17	63.67±4.51	31.67±6.02	3.00±1.00	2.00±0.00	0.00±0.00

n = 3
Results expressed as Mean ± SD

3.11 Effect of Ciprofloxacin on Phospholipase A2 activity

Table 11 shows the effect of ciprofloxacin on phospholipase A2 activity. There was a significant increase in the absorbance of the sample with increasing concentration of ciprofloxacin hence an increase in the enzyme activity. The maximum enzyme activity for Ciprofloxacin 0.1ml, 0.2ml, 0.4ml, 0.6ml and 0.8ml were 22.17%, 29.72%, 41.51%, 57.08% and 73.58% respectively. Prednisolone followed a different trend with the enzyme activity decreasing with increasing concentration of prednisolone.

Table 11: Effect of Ciprofloxacin on Phospholipase A2 activity

Treatment	Drug volume(ml)	Normal saline (ml)	HRBC (ml)	Crude enzyme (ml)	$CaCl_2$(ml)	Absorbance(418nm)	Maximum Enzyme Activity %	% Inhibition
Control	--	1.8	0.2	0.2	0.2	0.212 ± 0.018^{g}		
Cipro	0.1	1.7	0.2	0.2	0.2	0.047 ± 0.002^{ab}	22.17	77.83
	0.2	1.6	0.2	0.2	0.2	0.063 ± 0.004^{c}	29.72	70.28
	0.4	1.4	0.2	0.2	0.2	0.088 ± 0.012^{d}	41.51	58.49
	0.6	1.2	0.2	0.2	0.2	0.121 ± 0.004^{e}	57.08	42.92
	0.8	1.0	0.2	0.2	0.2	0.156 ± 0.004^{f}	73.58	26.42
Predni	0.2	1.6	0.2	0.2	0.2	0.050 ± 0.007^{bc}	23.58	76.42
	0.4	1.4	0.2	0.2	0.2	0.042 ± 0.002^{b}	19.81	80.19

n = 3
Results expressed as Mean ± SD
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column

3.12 Effect of Lincomycin on Phospholipase A2 activity

Table 12 shows the effect of lincomycin on phospholipase A2 activity. There was a decrease in the absorbance of the sample with increasing concentration of lincomycin hence a decrease in enzyme activity. The maximum enzyme activity for lincomycin 0.1ml, 0.2ml, 0.4ml, 0.6ml and 0.8ml were 21.70%, 16.04%, 13.68%, 8.96% and 9.91% respectively. Prednisolone followed a similar trend with the enzyme activity decreasing with increasing concentration of prednisolone.

Table 12: Effect of Lincomycin on Phospholipase A2 activity

Treatment	Drug volume(ml)	Normal saline (ml)	HRBC (ml)	Crude enzyme (ml)	$CaCl_2$(ml)	Absorbance(418nm)	Maximum Enzyme Activity %	% Inhibition
Control	--	1.8	0.2	0.2	0.2	0.212 ± 0.018^{g}		
Lincomycin	0.1	1.7	0.2	0.2	0.2	0.046 ± 0.012^{d}	21.70	78.30
	0.2	1.6	0.2	0.2	0.2	0.034 ± 0.006^{bcd}	16.04	83.96
	0.4	1.4	0.2	0.2	0.2	0.029 ± 0.002^{abc}	13.68	86.32
	0.6	1.2	0.2	0.2	0.2	0.019 ± 0.004^{a}	8.96	91.04
	0.8	1.0	0.2	0.2	0.2	0.021 ± 0.004^{ab}	9.91	90.09
Prednisolone	0.2	1.6	0.2	0.2	0.2	0.050 ± 0.007^{ef}	23.58	76.42
	0.4	1.4	0.2	0.2	0.2	0.042 ± 0.002^{e}	19.81	80.19

n = 3
Results expressed as Mean ± SD
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column.

3.13 Effect of Erythromycin on Phospholipase A2 activity

Table 13 shows the effect of erythromycin \on phospholipase A2 activity. There was an irregular increase in the absorbance of the samples with increasing concentration of erythromycin hence a similar trend in enzyme activity. The maximum enzyme activity for erythromycin 0.1ml, 0.2ml, 0.4ml, 0.6ml and 0.8ml were 17.92%, 28.77%, 17.45%, 37.36% and 69.33% respectively. The enzyme activity of the highest concentration of (0.8) was significantly higher than the enzyme activity of the lowest concentration of (0.1).

Table 13: Effect of Erythromycin on Phospholipase A2 activity

Treatment	**Drug volume(ml)**	**Normal saline (ml)**	**HRBC (ml)**	**Crude enzyme (ml)**	**$CaCl_2$(ml)**	**Absorbance(418nm)**	**Maximum Enzyme Activity %**	**% Inhibition**
Control	--	1.8	0.2	0.2	0.2	0.212 ± 0.018^{f}		
Erythromycin	0.1	1.7	0.2	0.2	0.2	0.038 ± 0.006^{a}	17.92	82.08
	0.2	1.6	0.2	0.2	0.2	0.061 ± 0.005^{b}	28.77	71.23
	0.4	1.4	0.2	0.2	0.2	0.037 ± 0.008^{a}	17.45	82.55
	0.6	1.2	0.2	0.2	0.2	0.079 ± 0.014^{d}	37.26	62.74
	0.8	1.0	0.2	0.2	0.2	0.147 ± 0.006^{e}	69.33	30.67
Prednisolone	0.2	1.6	0.2	0.2	0.2	0.050 ± 0.007^{c}	23.58	76.42
	0.4	1.4	0.2	0.2	0.2	0.042 ± 0.002^{c}	19.81	80.19

n = 3
Results expressed as Mean ± SD
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column

3.14 Effect of the various antibiotics on phospholipase A2 activity

Table 14 is one of comparison showing the effects of the various antibiotics on phospholipase A2 activity and how they compare to one another. The values of enzyme activity for the different concentrations of the test antibiotics and prednisolone were significantly ($p<0.05$) lower than those of the control. Enzyme activity increased concentration-wise with ciprofloxacin and erythromycin while it reduced with lincomycin and prednisolone.

Table 14: Effect of the various antibiotics on Phospholipase A2 activity (comparison)

Treatment	Drug volume(ml)	Normal saline (ml)	HRBC (ml)	Crude enzyme (ml)	$CaCl_2$(ml)	Absorbance(418nm)	Maximum Enzyme Activity %	% Inhibition
Control	--	1.8	0.2	0.2	0.2	0.212 ± 0.018^{f}		
Ciprofloxacin	0.1	1.7	0.2	0.2	0.2	0.047 ± 0.002^{de}	22.17	77.83
	0.2	1.6	0.2	0.2	0.2	0.063 ± 0.004^{f}	29.72	70.28
	0.4	1.4	0.2	0.2	0.2	0.088 ± 0.012^{g}	41.51	58.49
	0.6	1.2	0.2	0.2	0.2	0.121 ± 0.004^{h}	57.08	42.92
	0.8	1.0	0.2	0.2	0.2	0.156 ± 0.004^{i}	73.58	26.42
Lincomycin	0.1	1.7	0.2	0.2	0.2	0.046 ± 0.012^{d}	21.70	78.30
	0.2	1.6	0.2	0.2	0.2	0.034 ± 0.006^{bcd}	16.04	83.96
	0.4	1.4	0.2	0.2	0.2	0.029 ± 0.002^{abc}	13.68	86.32
	0.6	1.2	0.2	0.2	0.2	0.019 ± 0.004^{a}	8.96	91.04
	0.8	1.0	0.2	0.2	0.2	0.021 ± 0.004^{ab}	9.91	90.09
Erythromycin	0.1	1.7	0.2	0.2	0.2	0.038 ± 0.006^{cde}	17.92	82.08
	0.2	1.6	0.2	0.2	0.2	0.061 ± 0.005^{f}	28.77	71.23
	0.4	1.4	0.2	0.2	0.2	0.037 ± 0.008^{cde}	17.45	82.55
	0.6	1.2	0.2	0.2	0.2	0.079 ± 0.014^{g}	37.26	62.74
	0.8	1.0	0.2	0.2	0.2	0.147 ± 0.006^{i}	69.33	30.67
Prednisolone	0.2	1.6	0.2	0.2	0.2	0.050 ± 0.007^{ef}	23.58	76.42
	0.4	1.4	0.2	0.2	0.2	0.042 ± 0.002^{e}	19.81	80.19

n = 3
Results expressed as Mean ± SD
Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column

3.15 Effect of Ciprofloxacin on platelet aggregation

Table 15 shows the effect of the antibiotic, ciprofloxacin, on platelet aggregation. The absorbance of the control was significantly lower than the absorbance of the varying concentrations of ciprofloxacin. The absorbance of samples with ciprofloxacin was higher at 0 second than at 120 seconds. There was a stepwise decrease in the absorbance from 0 seconds through to 120 seconds. This was the same for all concentrations of the samples with ciprofloxacin and the normal control. Indomethacin showed a different pattern. There was a sharp decrease in the absorbance at around 30 seconds followed by a continuous increase up to 120 seconds. This was observed at all concentrations of the reference drug. The control without $CaCl_2$ had a very high absorbance when compared to the normal control samples, samples with ciprofloxacin and samples with indomethacin.

Table 15. Effect of Ciprofloxacin on platelet aggregation

GROUP	Drug (ml)	Normal saline (ml)	Absorbance (520nm)				
			0 sec	30 sec	60 sec	90 sec	120 sec
Control		1.8	0.322±0.048	0.319±0.046	0.317±0.045	0.314±0.045	0.313±0.044
Cipro	0.1	1.7	0.339±0.031	0.330±0.029	0.319±0.020	0.310±0.017	0.304±0.015
	0.2	1.6	0.329±0.006	0.330±0.009	0.317±0.006	0.309±0.009	0.303±0.013
	0.4	1.4	0.333±0.005	0.328±0.007	0.316±0.005	0.305±0.004	0.300±0.007
	0.6	1.2	0.327±0.010	0.324±0.005	0.311±0.006	0.302±0.012	0.298±0.003
	0.8	1.0	0.333±0.006	0.321±0.002	0.308±0.010	0.300±0.007	0.294±0.005
Indo	0.2	1.6	0.544±0.006	0.522±0.004	0.532±0.009	0.550±0.004	0.561±0.002
	0.4	1.4	0.523±0.009	0.511±0.010	0.606±0.006	0.607±0.005	0.614±0.009
	0.6	1.2	0.549±0.003	0.527±0.006	0.584±0.018	0.598±0.014	0.606±0.010
Control(no CaCl2)		1.8	0.490±0.010	0.492±0.007	0.486±0.003	0.488±0.009	0.489±0.006

n = 3
Results expressed as Mean ± SD

3.16 Effect of Erythromycin on platelet aggregation

Table 16 shows the effect of the antibiotic, erythromycin, on platelet aggregation. The absorbance of the control was significantly lower than the absorbance of the varying concentrations of erythromycin. The absorbance of samples with lincomycin was higher at 0 second than at 120 seconds. There was a stepwise decrease in the absorbance from 0 second through to 120 seconds. This was the same for all concentrations of the samples with erythromycin and the normal control. Indomethacin showed a different pattern. There was a sharp decrease in the absorbance at around 30 seconds followed by a continuous increase up to 120 seconds. This was observed at all concentrations of the reference drug. The control without $CaCl_2$ had a very high absorbance when compared to the normal control samples, samples with erythromycin and samples with indomethacin.

Table 16: Effect of Erythromycin on platelet aggregation

GROUP	Drug (ml)	Normal saline (ml)	Absorbance (520nm)				
			0 sec	30 sec	60 sec	90 sec	120 sec
Control		1.8	0.322±0.048	0.319±0.046	0.317±0.045	0.314±0.045	0.313±0.044
Erythro	0.1	1.7	0.331±0.010	0.324±0.006	0.315±0.003	0.309±0.004	0.300±0.011
	0.2	1.6	0.328±0.006	0.320±0.003	0.314±0.002	0.309±0.004	0.300±0.006
	0.4	1.4	0.326±0.005	0.319±0.002	0.311±0.003	0.305±0.007	0.301±0.008
	0.6	1.2	0.322±0.007	0.314±0.004	0.312±0.002	0.302±0.008	0.294±0.005
	0.8	1.0	0.319±0.006	0.311±0.003	0.305±0.005	0.300±0.005	0.294±0.003
Indo	0.2	1.6	0.544±0.006	0.522±0.004	0.532±0.009	0.550±0.004	0.561±0.002
	0.4	1.4	0.523±0.009	0.511±0.010	0.606±0.006	0.607±0.005	0.614±0.009
	0.6	1.2	0.549±0.003	0.527±0.006	0.584±0.018	0.598±0.014	0.606±0.010
Control(no CaCl2)		1.8	0.490±0.010	0.492±0.007	0.486±0.003	0.488±0.009	0.489±0.006

n = 3
Results expressed as Mean ± SD

3.17 Effect of Lincomycin on platelet aggregation

Table 17 shows the effect of the antibiotic, lincomycin, on platelet aggregation. The absorbance of the control was significantly lower than the absorbance of the varying concentrations of lincomycin. The absorbance of samples with erythromycin was lower at 0 second than at 120 seconds. There was a sharp decrease in the absorbance at around 30 seconds followed by a continuous increase up to 120 seconds. Indomethacin showed a similar pattern. The control without $CaCl_2$ had a very high absorbance when compared to the normal control samples, samples with lincomycin and samples with indomethacin.

Table 17: Effect of Lincomycin on platelet aggregation

GROUP	Drug (ml)	Normal saline (ml)	Absorbance (520nm)				
			0 sec	30 sec	60 sec	90 sec	120 sec
Control		1.8	0.322±0.048	0.319±0.046	0.317±0.045	0.314±0.045	0.313±0.044
Linco	0.1	1.7	0.313±0.005	0.311±0.006	0.322±0.003	0.334±0.005	0.350±0.002
	0.2	1.6	0.310±0.015	0.313±0.017	0.323±0.020	0.339±0.005	0.344±0.002
	0.4	1.4	0.320±0.005	0.314±0.012	0.329±0.017	0.340±0.004	0.353±0.004
	0.6	1.2	0.341±0.005	0.315±0.023	0.351±0.020	0.366±0.006	0.365±0.003
	0.8	1.0	0.333±0.018	0.317±0.018	0.357±0.005	0.357±0.009	0.357±0.002
Indo	0.2	1.6	0.544±0.006	0.522±0.004	0.532±0.009	0.550±0.004	0.561±0.002
	0.4	1.4	0.523±0.009	0.511±0.010	0.606±0.006	0.607±0.005	0.614±0.009
	0.6	1.2	0.549±0.003	0.527±0.006	0.584±0.018	0.598±0.014	0.606±0.010
Control(no CaCl2)		1.8	0.490±0.010	0.492±0.007	0.486±0.003	0.488±0.009	0.489±0.006

n = 3
Results expressed as Mean ± SD

3.18 Effect of the various antibiotics on platelet aggregation

Table 18 shows the effect of the various antibiotics on platelet aggregation. Ciprofloxacin, erythromycin and the normal control all followed a similar trend. They all had stepwise increase in absorbance from time 0 second through to time 120seconds. The pattern for lincomycin differed from that of the other two drugs. There was a sharp decrease in the absorbance at around 30 seconds followed by a continuous increase up to 120 seconds and this was similar to what was obtained from indomethacin.

Table 18: Effect of the various antibiotics on platelet aggregation (comparative)

GROUP	Drug (ml)	Normal saline (ml)	Absorbance (520nm)				
			0 sec	30 sec	60 sec	90 sec	120 sec
Control		1.8	0.322±0.048	0.319±0.046	0.317±0.045	0.314±0.045	0.313±0.044
Cipro	0.1	1.7	0.339±0.031	0.330±0.029	0.319±0.020	0.310±0.017	0.304±0.015
	0.2	1.6	0.329±0.006	0.330±0.009	0.317±0.006	0.309±0.009	0.303±0.013
	0.4	1.4	0.333±0.005	0.328±0.007	0.316±0.005	0.305±0.004	0.300±0.007
	0.6	1.2	0.327±0.010	0.324±0.005	0.311±0.006	0.302±0.012	0.298±0.003
	0.8	1.0	0.333±0.006	0.321±0.002	0.308±0.010	0.300±0.007	0.294±0.005
Erythro	0.1	1.7	0.331±0.010	0.324±0.006	0.315±0.003	0.309±0.004	0.300±0.011
	0.2	1.6	0.328±0.006	0.320±0.003	0.314±0.002	0.309±0.004	0.300±0.006
	0.4	1.4	0.326±0.005	0.319±0.002	0.311±0.003	0.305±0.007	0.301±0.008
	0.6	1.2	0.322±0.007	0.314±0.004	0.312±0.002	0.302±0.008	0.294±0.005
	0.8	1.0	0.319±0.006	0.311±0.003	0.305±0.005	0.300±0.005	0.294±0.003
Linco	0.1	1.7	0.313±0.005	0.311±0.006	0.322±0.003	0.334±0.005	0.350±0.002
	0.2	1.6	0.310±0.015	0.313±0.017	0.323±0.020	0.339±0.005	0.344±0.002
	0.4	1.4	0.320±0.005	0.314±0.012	0.329±0.017	0.340±0.004	0.353±0.004
	0.6	1.2	0.341±0.005	0.315±0.023	0.351±0.020	0.366±0.006	0.365±0.003
	0.8	1.0	0.333±0.018	0.317±0.018	0.357±0.005	0.357±0.009	0.357±0.002
Indo	0.2	1.6	0.544±0.006	0.522±0.004	0.532±0.009	0.550±0.004	0.561±0.002
	0.4	1.4	0.523±0.009	0.511±0.010	0.606±0.006	0.607±0.005	0.614±0.009
	0.6	1.2	0.549±0.003	0.527±0.006	0.584±0.018	0.598±0.014	0.606±0.010
Control(no CaCl2)		1.8	0.490±0.010	0.492±0.007	0.486±0.003	0.488±0.009	0.489±0.006

n = 3
Results expressed as Mean ± SD

CHAPTER FOUR

DISCUSSION

In this study, the anti-inflammatory activity of three antibiotics and their ability to reduce the production of pro-inflammatory mediators have been investigated both *in vivo* and *in vitro*. Rat paw oedema was used as a model of acute inflammation and the inflammatory reaction was induced in normal animals. The antibiotics were administered, orally, 1 hour before the agar challenge. Erythromycin had minimal effect on the rat paw oedema with a non-significant ($p>0.05$) inhibition with reference to time. Ciprofloxacin and lincomycin supressed the agar-induced rat paw oedema both at the early and later phases of oedema. The oedema reduction by these two antibiotics was slightly better when compared to that obtained for indomethacin, the standard drug used in this test. The formation of oedema is as a result of the interactive action of pro-inflammatory mediators such as serotonin, histamine and bradykinin at the site of injury leading to an increase in the permeability of the vesicles and blood flow (Kang *et al*., 2008). The formation of oedema by agar occurs in two distinct phases. The first phase starts at 0 time (agar induction) and could last till the second hour. It is caused by the release of the mediators, serotonin and histamine. The second phase is as a result of the release of bradykinin, protease, prostaglandins and lysosomes. It commences from the 3rd hour post-induction to the 5th hour (Wallace, 2002). The injection of agar brings about the changes in vascular structure resulting in the increased movement of fluid and white blood cells (especially neutrophils) into the affected area (Yankanchi and Koli, 2010). The reduction of oedema formation in the first phase expressed by both ciprofloxacin and lincomycin in this work showed that both drugs were effective in suppressing the release or action of phase one (1) mediators to arrive at the site of injury, thus inhibiting oedema by reducing vascular permeability and fluid transudation. Erythromycin showed no inhibitory effect on the release or action of phase one (1) mediators. In the second phase, all three antibiotics suppressed oedema. The reduction of the oedema from the 3rd hour to the 5th hour was significant ($p<0.05$) with lincomycin and non-significant ($p>0.05$) for erythromycin when compared with control. This also indicated that lincomycin was effective in suppressing the release and activity of bradykinin, proteases, prostaglandins or lysosomes. Ciprofloxacin and erythromycin were not as effective as lincomycin in suppressing oedemogenesis. This result revealed that the intake of these three antibiotics (especially lincomycin and ciprofloxacin) could offer protection against inflammatory oedema

and thus could also be used when treating and managing conditions of inflammation. Normally, non-steroidal anti-inflammatory drugs (NSAIDs) possess anti-inflammatory and analgesic activities probably acting via the same mechanism. They do this mainly by inhibiting prostaglandin formation (Vane, 1971). These prostaglandins are known to cause pain (Roberts and Morrow, 2001) and sensitise the skin to painful stimuli (Dray, 1995). Prostaglandins are derived from arachidonic acids, products of phospholipase A_2 activity (Foegh and Ramwell, 2001). The enzyme, phospholipase A_2, cleaves arachidonic acid (20-carbon fatty acid) from membrane-bound phospholipids. The enzyme activity in this study was assayed using its action on the membrane of erythrocytes where it created an outflow thereby causing haemoglobin to escape into the medium. High haemoglobin concentrations revealed high enzyme activity hence the increase in absorbance since haemoglobin absorbs best at 418nm. Lincomycin suppressed the action of the enzyme with increasing concentration. It might achieve this in the same manner anti-inflammatory steroids work. These steroids induce lipocortin which inhibits phospholipase A_2 (Schimmer and Parker, 2001). The actions of erythromycin and ciprofloxacin are not understood but both drugs best inhibited the enzyme at their lowest concentrations. The analgesic effect of ciprofloxacin might be derived from a possible interaction with central nociceptors. The hot plate method was used to induce pain by thermal stimulus and this pain was specific for centrally mediated nociception (Parkhouse and Pleuvry, 1979). NSAIDs have analgesic effects and these are known to be as a result of action on the central and peripheral neurons (Konttiene *et al*., 1994; Gerbhart and McCormack, 1994). Lincomycin and erythromycin did not show analgesic effects on the thermal-induced pain indicating that both drugs have no effect on the neurons and thus cannot be used to relieve pain. From the result in Table 10, there was a dose-dependent decrease in leucocyte mobilization upon treatment with all three antibiotics. The agar suspension was able to cause an injury which was responded to by the drugs, 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 numbers of neutrophils and other phagocytic cells increased during injury and were directly responsible for the high number of leukocytes that were mobilized to the site of an injury (Vega and Corbi, 2006). 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 (Dale *et al*., 2008). Indomethacin used in this study decreased the mobilization of leukocytes and this is in line with Bhattacherjee *et al*, (1983) that stated that high doses of indomethacin inhibited the accumulation of leukocytes. In

the platelet aggregation test, $CaCl_2$caused an increase in calcium concentration in the test-tube. This would cause a number of structural and functional changes of the platelet in the platelet-rich plasma (PRP). The increased calcium concentration could also stimulate phospholipase $A_2$to metabolize thromboxane A_2which is a potent activator of platelets. The combined effect brings about shape changes which allow the platelets to interact with one another to form aggregates. As platelets aggregate, the light transmission of the sample increases hence the absorbance decreases. Ciprofloxacin and erythromycin showed a stepwise decrease in absorbance from time zero sec through to time 120 sec when $CaCl_2$ was added. This showed that both drugs had no effect on platelet aggregation. Lincomycin caused a decrease in absorbance when $CaCl_2$was added but showed a sharp increase from around 30 seconds through to 120 seconds. This trend was similar to that of indomethacin. This showed that the addition of $CaCl_2$ caused the platelets to aggregate but the presence of the drug, lincomycin, stopped this and possibly prevented further shape change and aggregation hence the increase in absorbance.

4.2 Conclusion

The present study revealed that these antibiotics had anti-inflammatory activity, which probably depended on their ability to prevent the production of some pro-inflammatory mediators and cytokines. It indicated that these agents, could exert other therapeutic effects independent of their antibacterial activity. Lincomycin had higher effect on the paw oedema, phospholipase A2 and platelet aggregation tests. Ciprofloxacin lightly inhibited paw oedema but helped reduce reaction latency to pain and could be used as an analgesic. Erythromycin inhibited paw oedema especially at the second stage. All three prevented leukocyte mobilization. No antibiotic showed total anti-inflammatory activity though. Despite the fact that there were non-significant differences between the maximum effects of the test drugs and the reference drugs, these test drugs actually had these effects to an extent. Utilizing these findings, these antibiotics can actually be drafted for use as anti-inflammatory and analgesic drugs. More work could be done on other inflammatory parameters. The parameters include membrane stabilisation, vascular permeability and effect on prostaglandin synthase among others.

REFERENCES

Agen, C., Danesi, R., Blandizzi, C., Costa, M., Stacchini, B., Favini, P. and Del Tacca, M. (1993). Macrolide antibiotics as anti-inflammatory agents: Roxithromycin in an unexpected role. *Agents Actions,* **38**:85–90.

Andersen, H.H., Elberling, J. and Arendt-Nielsen, L. (2015). "Human surrogate models of histaminergic and non-histaminergic itch". *Acta Dermato-Venereologica,* **22**:18-21.

Andre, P., Prasad, K.S., Denis, C.V., He, M., Papalia, J.M., Hynes, R.O., Philips, D.R. and Wagner, D.D. (2002). CD40L stabilizes arterial thrombi by a beta3 integrin dependent mechanism. *Nature Medicine*, **8**:247-252.

Andreas, K., Elisabeth, W., Kathryn, J.W. and Peter, N.M. (2013). Complement Peptide C5a, C4a, and C3a Receptors. *Pharmacological Reviews,* **65**(1): 500–543.

Anosike, C. A., Obidoa, O. and Ezeanyika, L. U. S. (2012). The anti-inflammatory activity of garden egg, *Solanium aethiopicum*, on egg albumin-induced oedema and granuloma tissue formation in rats. *Asian Pacific Journal of Tropical Medicine,* **1**:62-66.

Bajzar, L., Morser, J. and Nesheim, M. (1996). TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex. *The Journal of Biological Chemistry*, **271**(28): 16603–8.

Ball P. (1986): Ciprofloxacin: an overview of adverse experiments. *Journal of Antimicrobial Chemotherapy*, **18**:187–193.

Barland, C.O., Zettersten, E., Brown, B.S., Ye, J., Elias, P.M. and Ghadially, R. (2004). Imiquimod-induced interleukin-1 alpha stimulation improves barrier homeostasis in aged murine epidermis. *The Journal of Investigative Dermatology,* **122**(2): 330–6.

Bas, M., Adams, V., Suvorava, T., Niehues, T., Hoffmann, T.K. and Kojda, G. (2007). Nonallergic Angioedema: Role of Bradykinin. *Allergy,* **62**(8): 842–56.

Bankers-Fulbright, J.L., Kalli, K.R. and McKean, D.J. (1996). Interleukin-1 signal transduction. *Life Sciences,* **59**(2): 61–83.

Berg, J. (1988): Clinical indications for enrofloxacin in domestic animals and poultry. In: Quinolones: A new class of antimicrobial agents for use in veterinary medicine. In: Proc. West Vet. Conf, Las Vegas, Nevada: Mobay Corporation.

Bezerra, J.A., Bugge, T.H., Melin-Aldana, H., Sabla, G., Kombrinck, K.W., Witte, D.P. and Degen, J.L. (1999). Plasminogen deficiency leads to impaired remodelling after a toxic

injury to the liver. *Proceedings of theNational Academy of Sciences U.S.A.* **96**(26): 15143–8.

Bhattacherjee, P., Williams, R. N. and Eakins, K. E. (1983). A comparison of the ocular anti-inflammatory activity of steroidal and non-steroidal compounds in the rat. *Investigative Ophthalmology and Visual Science,* **24**: 1143-1146.

Böhm, I., Speck, U. and Schild, H.H. (2011). Pilot study on basophil activation induced by contrast medium. *Fundamental Clinical Pharmacology,* **25**(2): 267–76.

Borissoff, J.I., Spronk, H.M., Heeneman, S. and ten Cate, H (2009). Is thrombin a key player in the coagulation-atherogenesis maze? *Cardiovascular Research,* **82**(3): 392–403.

Borissoff, J.I., Heeneman, S., Kilinç, E., Kassák, P., Van Oerle, R., Winckers, K., Govers-Riemslag, J.W., Hamulyák, K., Hackeng, T.M., Daemen, M.J., ten Cate, H. and Spronk, H.M. (2010). Early atherosclerosis exhibits an enhanced procoagulant state. *Circulation,* **122**(8): 821–30.

Borissoff, J.I., Spronk, H.M. and ten Cate, H. (2011). The haemostatic system as a modulator of atherosclerosis. *The New England Journal of Medicine,* **364**(18): 1746–60.

Bryskier, A., Agouridas, C. and Chantot, J.F. (1994). New medical targets for macrolides. *Expert Opinion on Investigational Drugs,* **3**:405–410.

Buer, J. K. (2014). Origins and impact of the term 'NSAID'. *Inflammopharmacology*, **22**(5): 263-267.

Burns, A. R., Walker, D. C., Brown, E. S., Thurmon L. T., Bowden, R. A., Keese, C. R., Simon, S. I., Entman, M. L. and Smith, C. W. (1997). Neutrophil transendothelial migration is independent of tight junctions and occurs preferentially at tricellular corners. *Journal of Immunology*, **159**:2893-903.

Carlier, M.B., Scorneaux, B., Zenebergh, A., Desnottes, J. F. and Tulkens, P.M. (1990). Cellular uptake, localization and activity of fluoroquinolones in uninfected and infected macrophages. *Journal of Antimicrobial Chemotherapy*, **26**:27–39.

Chapman, J.S. and Georgopapadakou, N.H. (1988). Routes of quinolone permeation in E. coli. *Antimicrobial Agents and Chemotherapy*, **32**:438–442.

Chu, D., Fernandez, P., Claiborne, A., Shen, L. and Pernet, A. (1988). Structure-activity relationships in quinolone antimicrobials: design, synthesis and biological activities of novel isothiazoquinolones. *Drugs under Experimental and Clinical Research*, **14**:379–383.

Cinamon, G., Shinder, V. and Alon, R. (2001). Shear forces promote lymphocyte migration across vascular endothelium bearing apical chemokines. *Nature Immunology*, **2**:515-22.

Cohen, S. and Burns, R.C. (2002). Pathways of the Pulp (8th Ed.). St. Louis, Mosby. p. 465.

Cotran, R. S., Kumar, V. and Collins, T. (1999). Robbins *Pathologic Basis of Disease*. Philadelphia: W.B Saunders Company

da Silva, E. Z., Jamur, M. C. and Oliver, C. (2014). Mast cell function: a new version of an old cell. *Journal of Histochemistry & Cytochemistry*, **62**(10): 698-738.

Dale, D. C., Boxer, L. and Liles, C. W. (2008). The phagocytes: neutrophils and monocytes. *Blood*, **112**: 935-945.

Dale, H.H. and Laidlaw, P.P. (1910). The physiological action of beta-iminazolylethylamine. *The Journal of Physiology*, **41**(5): 318–44.

Dalhoff, A., Ambrose, P. G. and Mouton, J. W. (2009). A long journey from minimum inhibitory concentration testing to clinically predictive breakpoints: deterministic and probabilistic approaches in deriving breakpoints. *Infection,* **37** (4): 296–305.

Dalziel, K., Dykes, P.J. and Marks, R. (1987). The effect of tetracycline and erythromycin in a model of acne-type inflammation. *British Journal of Experimental Pathology,* **68**:67–70.

Dangerfield, J., Larbi, K. Y., Huang, M. T., Dewar, A. and Nourshargh, S. (2002). PECAM-1 (CD31) homophilic interaction up-regulates α6β1 on transmigrated neutrophils in vivo and plays a functional role in the ability of α6 integrins to mediate leukocyte migration through the perivascular basement membrane. *The Journal of Experimental Medicine*, **196**:1201-12.

Daniel, J.L., Molish, I.R., Rigmaiden, M. and Stewart, G. (1984). Evidence for a role of myosin phosphorylation in the initiation of the platelet shape chane response. *The Journal of Biological Chemistry*, **259**:9826-9831.

Dardick, C. and Ronald, P. (2006). Plant and animal pattern recognition receptors signal through

De Larco, J.E., Wuertz, B.R. and Furcht, L.T. (2004). The Potential Role of Neutrophils in Promoting the Metastatic Phenotype of Tumors Releasing Interleukin-8. *Clinical Cancer Research,* **10**(15): 4895–900.

Degen, S.J. and Davie, E.W. (1987). Nucleotide sequence of the gene for human prothrombin. *Biochemistry*, **26**(19): 6165–77.

Dendorfer, A., Wolfrum, S., Wagemann, M., Qadri, F. and Dominiak, P. (2001). Pathways of bradykinin degradation in blood and plasma of normotensive and hypertensive rats. *American Journal Physiology – Heart and Circulatory Physiology*, **280**(5): 2182–8.

Dessy, C. and Ferron, O. (2004). Pathophysiological Roles of Nitric Oxide: In the Heart and the Coronary Vasculature. *Current Medical Chemistry – Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry,* **3** (3): 207–216.

Di Giuseppe, M. (2003). Nelson Biology 12. Toronto: Thomson Canada. **p**. 473. ISBN 0-17-625987-2.

Dinarello, C.A. (1997). Induction of interleukin-1 and interleukin-1 receptor antagonist. *Seminars in Oncology,* **24**(3): 981–993.

Diver, J.M. and Wise, R. (1986). Morphological and biochemical changes in Escherichia coli after exposure to ciprofloxacin. *Journal of Antimicrobial Chemotherapy*, **18**:31–41.

Dray, A. (1995). Inflammatory mediators of pain. *British Journal of Anaesthesia,* **75**: 125-131.

Ear, T. and McDonald, P.P. (2008). Cytokine generation, promoter activation, and oxidant-independent NF-kappaB activation in a transfectable human neutrophilic cellular model. *BMC Immunology*, **9**:14.

Ekwueme, F. N., Nwodo, O. F. C., Joshua, P. E., Nkwocha, C. and Eluka, P. E. (2015). Qualitative and Quantitative Phytochemical Screening of the Aqueous Leaf Extract of *Senna mimosoides*: its effects in in vivo leukocyte mobilization induced by inflammatory stimulus. *International Journal of Current Microbiology and Applied Sciences,* **4**(5): 1176-1188.

Erickson, A. C. and Couchman, J. R. (2000). Still more complexity in mammalian basement membranes. Journal of Histochemistry and Cytochemistry, 48:1291-306.

Ermert, D., Niemiec, M.J., Röhm, M., Glenthoj, A., Borregard, N. and Urban, C.F. (2013). Candida albicans escapes mouse neutrophils. *Journal of Leukocyte Biology*, **12**:4-7.

Escolar, G., Krumwiede, M. and White, J.G. (1986). Organization of the actin cytoskeleton of resting and activated platelets in suspension. *The American Journal of Pathology*, **123**: 86-94.

Feldmann, M. and Saklatvala, J. (2001). Proinflammatory cytokines. In Durum, S.K., Oppenheim, J.J. and Feldmann, M. Cytokine reference: A compendium of cytokines and other mediators of host defence. Boston: Academic Press. **pp**. 291–306.

Feng, D., Nagy, J. A., Pyne, K., Dvorak, H. F. and Dvorak, A. M. (1998). Neutrophils emigrate from venules by a transendothelial cell pathway in response to FMLP. *The Journal of Experimental Medicine*, **187**:903-15.

Ferrero-Miliani L., Nielsen, O. H., Andersen, P. S., Girardin, S. E. (2007). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. *Clinical and Experimental Immunology*, **147**(2): 06-11.

Fernandes, P.B. (1988). Mode of action, and in vitro and in vivo activities of the fluoroquinolones. *The Journal of Clinical Pharmacology*, **28**:156–168.

Fitzgeorge, R., Featherstone, A. and Baskerville, A. (1988). The effect of ofloxacin on the intracellular growth of *Legionella pneumophila* in guinea pig alveolar phagocytes. *Journal of Antimicrobial Agents and Chemotherapy*, **22**:53–57.

Foegh, M. L. and Ramwell, P. W. (2001). The eicosanoids: prostaglandins, thromboxanes, leukotrienes and related compounds. In (Kartzung, B. G. ed.). Basic and Clinical Pharmacology.8th edition. Lange Medical Books/McGraw-Hill, New York. 311-325.

Foerster, D. (1987). Visualization of the bactericidal action of Baytril by microphotography. *Veterinary Medicine Reviews*, **2**:100–103.

Forsgren, M., Råden, B., Israelsson, M., Larsson, K. and Hedén, L.O. (1987). Molecular cloning and characterization of a full-length cDNA clone for human plasminogen. *FEBS Letters,* **213**(2): 254–60.

Fox, J.E. (2001). Cytoskeletal proteins and platelet signalling. *Thrombosis and Haemostasis*, **86**:198-213.

Gahring, L.C., Buckley, A. and Daynes, R.A. (1985). Presence of epidermal-derived thymocyte activating factor/interleukin 1 in normal human stratum corneum. *The Journal of Clinical Investigation*, **76**(4): 1585–91.

Gebhart, G. F. and McCormack,K. J. (1994). Neuronal plasticity; implications for pain therapy. *Drugs*, **47**(5): 1-47.

Goodman, L.S. and Gilman, A. (1992). The Pharmacological Basis of Therapeutics. (8th ed.) New York, Macmillan. 1057.

Grattan, C.E., Dawn, G., Gibbs, S. and Francis, D.M. (2003). Blood basophil numbers in chronic ordinary urticaria and healthy controls: diurnal variation, influence of loratadine and prednisolone and relationship to disease activity. *Clinical and Experimental Allergy*, **33**(3): 337–41.

Green, S.J., Mellouk, S., Hoffman, S.L., Meltzer, M.S. and Nacy, C.A. (1990). Cellular mechanisms of nonspecific immunity to intracellular infection: Cytokine-induced synthesis of toxic nitrogen oxides from L-arginine by macrophages and hepatocytes. *Immunology Letters*, **25**(1–3): 15–9.

Green, S.J., Nacy, C.A., Schreiber, R.D., Granger, D.L., Crawford, R.M., Meltzer, M.S. and Fortier, A.H. (1993). Neutralization of gamma interferon and tumour necrosis factor alpha blocks in vivo synthesis of nitrogen oxides from L-arginine and protection against *Francisella tularensis* infection in *Mycobacterium bovis* BCG-treated mice. *Infection and immunity*, **61**(2): 689–98.

Green, S.J., Scheller, L.F., Marletta, M.A., Seguin, M.C., Klotz, F.W., Slayter, M., Nelson, B.J. and Nacy, C.A. (1994). Nitric oxide: Cytokine-regulation of nitric oxide in host resistance to intracellular pathogens. *Immunology Letters*, **43**(1–2): 87–94.

Green[b], S.J., Crawford, R.M., Hockmeyer, J.T., Meltzer, M.S. and Nacy, C.A. (1990). Leishmania major amastigotes initiate the L-arginine-dependent killing mechanism in IFN-gamma-stimulated macrophages by induction of tumour necrosis factor-alpha. *Journal of Immunology,* **145**(12): 4290–7.

Greenhalgh, D.G. (1998). The role of apoptosis in wound healing. *The International Journal of Biochemistry & Cell Biology,* **30**(9): 1019–1030.

Gryglewski, R.J. (2008). Prostacyclin among prostanoids. *Pharmacological Reports*, **60**:3-11.

Haake, S.K. and Huang, G.T.J. (2002). Molecular Biology of the host-Microbe Interaction in Periodontal Diseases (Selected Topics). In Newman, Takei, Carranza, editors: Clinical Periodontology, 9th Edition. Philadelphia: W.B.Saunders Co., page 162.

Hand, W.L., King-Thompson, N. and Holman, J.W. (1987). Entry of roxithromycin (RU 965) imipenem, cefotaxime, trimethoprim and metronidazole into human polymorphonuclear leukocytes. *Antimicrobial Agents and Chemotherapy*, **31**:1535–1537.

Hand, W.L., Hand, D.L. and King-Thompson, N.L. (1990). Antibiotic inhibition of the respiratory burst response in human polymorphonuclear leukocytes. *Antimicrobial Agents and Chemotherapy*, **34**:863–870.

Harada, A., Sekido, N., Akahoshi, T., Wada, T., Mukaida, N. and Matsushima, K. (1994). Essential involvement of interleukin-8 (IL-8) in acute inflammation. *Journal of Leukocyte Biology*, **56**(5): 559–64.

Hauser, C., Saurat, J.H., Schmitt, A., Jaunin, F. and Dayer, J.M. (1986). Interleukin 1 is present in normal human epidermis. *Journal of Immunology*, **136**(9): 3317–23.

Hedges, J.C., Singer, C.A. and Gerthoffer, W.T. (2000). Mitogen-activated protein kinases regulate cytokine gene expression in human airway myocytes. *American Journal of Respiratory Cell and Molecular Biology*, **23**(1): 86–94.

Hooper, D. and Wolfson, J. (1985). The fluoroquinolones: Structures, mechanisms of action and resistance and spectra of activity in vitro. *Antimicrobial Agents and Chemotherapy*, **28**:581–586.

Hou, Y.C., Janczuk, A. Wang, P.G. (1999). Current trends in the development of nitric oxide donors. *Current Pharmaceutical Design*, **5**(6): 417–41.

Iwueke, A. V., Nwodo, O. F. C. and Okoli, C. O. (2006). Evaluation of the anti-inflammatory and analgesic activities of *Vitex doniana* leaves. *African Journal of Biotechnology,* **5**(20): 1939-1935.

Jacobs, L., Nawrot, T.S., De Geus, B., Meeusen, R., Degraeuwe, B., Bernard, A., Sughis, M., Nemery, B. and Panis, L. (2010). Subclinical responses in healthy cyclists briefly exposed to traffic-related air pollution. *Environmental Health*, **9**(64): 64.

Jakubowski, H.V. and Owen, W.G. (1989). Macromolecular specificity determinants on thrombin for fibrinogen and thrombomodulin. *The Journal of Biological Chemistry*, **264**(19): 11117–21.

Janeway, C., Paul, T., Mark, W. and Mark, S. (2001). Immunobiology (5th Ed). New York and London: Garland Science. Pp 54-62.

Janeway, C. A.; et al. (2005). Immunobiology: the immune system in health and disease (6th ed.). New York: Garland Science. ISBN 0-8153-4101-6.

Janssen, B .J., Huizinga, E. G., Raaijmakers, H.C., Roos, A., Daha, M. R., Nilsson-Ekdahl, K., Nilsson, B. and Gros, P. (2005). Structures of complement component C3 provide insights into the function and evolution of immunity. *Nature*, **437**(7058): 505–11.

Janssen, P. A. J. and Jagneau, A. H. (1957). A new series of potent analgesics: Dextro 2,2-diphenyl-3 methyl-H-morholino-butryprolidine and related amides.. Part 1. Chemical structure and pharmacological activity. *Journal of pharmaceutical Sciences*, **9**:38

Jennings, L.K., Fox, J.E., Edwards, H.H. and Philips, D.R. (1981). Changes in the cytoskeletal structure of human platelets following thrombin activation. *The Journal of Biological Chemistry*, **256**:6927-6932.

Jia, T. and Pamer, E.G. (2009). Dispensable but not Irrelevant. *Science,* **325**(5940): 549–550.

Joseph, P. E. (2008). "Steroids and Warfarin Therapy". Coumarin Anticoagulant Research Progress. Nova Publishers, 2008. p. 18.

Kamijo, R., Gerecitano, J., Shapiro, D., Green, S.J., Aguet, M., Le, J. and Vilcek, J. (1995). Generation of nitric oxide and clearance of interferon-gamma after BCG infection are impaired in mice that lack the interferon-gamma receptor. *Journal of Inflammation*, **46**(1): 23–31.

Kang, H. S., Lee, J. Y. and Kim, C. J. (2008). Anti-inflammatory activity of arctigenin from *Forsythiae fructus*. *Journal of Ethnopharmacology*, **116**:305-310.

Kearney, P. M., Baigent, C., Godwin, J., Halls, H., Emberson, J. R. and Patrono, C. (2006). Do selective cyclooxygenase-2 inhibitors and traditional nonsteroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. *British Medical Journal*, **332**(7553): 1302-1308.

Kitayama, J., Hidemura, A., Saito, H. and Nagawa, H. (2000). Shear stress affects migration behavior of polymorphonuclear cells arrested on endothelium. *Cellular Immunology*, **203**:39-46.

Konno, S., Asano, K., Kurokawa, M., Ikeda, K., Okamoto, K. and Adachi, M. (1994). Anti-asthmatic activity of a macrolide antibiotic, roxithromycin: Analysis of possible mechanisms in vitro and in vivo. *International Archives of Allergy and Immunology,* **105**:308–316.

Konttiene, Y. T., Kemppinen, P., Segerberg, M., Hukkanen, M., Rees, R., Santavirta, S., Sorsa, T., Pertovaara, A. and Polak, J. M. (1994). Peripheral and spinal neural mechanisms in arthritis with particular reference to treatment of inflammation and pain. *Arthritis and Rheumatology*, **37**:965-982.

Krombach, F., Münzing, S., Allmeling, A.M., Gerlach, J. T., Behr, J. and Dörger, M. (1997). Cell size of alveolar macrophages: an interspecies comparison. *Environmental Health Perspectives,* **105**: 1261–3.

Labro, M.T., El Benna, J. and Babin-Chevaye, C. (1989). Comparison of the in vitro effect of several macrolides on the oxidative burst of human neutrophils. *Journal of Antimicrobial Chemotherapy*, **24**:561–572.

Larbi, K. Y., Allen, A. R., Tam, F. W., Haskard, D. O., Lobb, R. R., Silva, P. M. and Nourshargh, S. (2000). VCAM-1 has a tissue-specific role in mediating interleukin-4-induced eosinophil accumulation in rat models: evidence for a dissociation between endothelial-cell VCAM-1 expression and a functional role in eosinophil migration. *Blood*, **96**:3601-9.

Larbi, K. Y., Dangerfield, J. P., Culley, F. J., Marshall, D., Haskard, D. O., Jose, P. J., Williams, T. J. and Nourshargh, S. (2003). P-selectin mediates IL-13-induced eosinophil transmigration but not eotaxin generation in vivo: a comparative study with IL-4-elicited responses. *Journal of Leukocyte Biology*, **73**:65-73.

Laufen, H., Wildfeuer, A. and Rader, K. (1985). Uptake of antimicrobial agents by human polymorphonuclear leucocytes. *Drug Research,* **35**:1097–1099.

Law, R.H., Caradoc-Davies, T., Cowieson, N., Horvath, A.J., Quek, A.J., Encarnacao, J.A., Steer, D., Cowan, A., Zhang, Q., Lu, B.G., Pike, R.N., Smith, A.I., Coughlin, P.B. and Whisstock, J.C. (2012). The X-ray crystal structure of full-length human plasminogen. *Cell Reports*, **1**(3): 185–90.

Ma, Y.Q., Qin, J. and Plow, E.F. (2007). Platelet integrin alpha(IIb)beta(3): activation systems. *Journal of Thrombosis and Haemostasis*, **5**:1345-1352.

Manthey, H.D., Woodruff, T.M., Taylor, S.M. and Monk, P.N. (2009). Complement component 5a (C5a). *The International Journal of Biochemistry & Cell Biology*, **41**(11): 2114–7.

March, C.J., Mosley, B., Larsen, A., Cerretti, D.P., Braedt, G., Price, V., Gillis, S., Henney, C.S., Kronheim, S.R. and Grabstein, K. (1985). Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature*, **315**(6021): 641–7.

Marieb, E. (2001). Human Anatomy & Physiology. San Francisco: Benjamin Cummings. p. 414.

Martínek, J., Hlavova, K. and Zavada, F. (2010). A surviving myth-corticosteroids are still considered ulcerogenic by a majority of physicians. *Scandinavian Journal of Gastroenterology*, **45**(10): 1156-1161.

Mellouk, S., Green, S.J., Nacy, C.A., Hoffman, S.L. (1991). IFN-gamma inhibits development of Plasmodium berghei exoerythrocytic stages in hepatocytes by an L-arginine-dependent effector mechanism. *Journal of Immunology*, **146**(11): 3971–6.

Mikasa, K., Kita, E., Sawaki, M., Kunimatsu, M., Hamada, K., Konishi, M., Kashiba, S. and Narita, N. (1992). The anti-inflammatory effect of erythromycin in zymosan-induced peritonitis of mice. *Journal of Antimicrobial Chemotherapy*, **30**:339–348.

Mills, C.D. (2012). M1 and M2 Macrophages: Oracles of Health and Disease. *Critical Reviews in Immunology,* **32**(6): 463–488.

Miyata, T., Iwanaga, S., Sakata, Y., Aoki, N. (1982). Plasminogen Tochigi: inactive plasmin resulting from replacement of alanine-600 by threonine in the active site. *Proceedings of the National Academy of Sciences U.S.A.*, **79**(20): 6132–6.

Montay, G., Goueffon, Y. and Roquet, F. (1984). Absorption, distribution, metabolic fate and elimination of pefloxacin mesylate in mice, rats, dogs, monkeys and humans. *Antimicrobial Agents and Chemotherapy,* **25**:463–472.

Mutschler, E. and Schäfer-Korting, M. (1997). Arzneimittelwirkungen (in German) (7 Ed.). Stuttgart: Wissenschaftliche Verlagsgesellschaft.

Nakanishi, Kenji. (2010). Basophils as APC in Th2 response in allergic inflammation and parasite infection. *Current Opinion in Immunology*, **22**(6): 814–820.

Nanda, N., Andre, P., Bao, M., Clauser, K., Deguzman, F., Howie, D., Conley, P.B., Terhost, C. and Philips, D.R. (2005). Platelet aggregation induces platelet aggregate stability via SLAM family receptor signalling. *Blood*, **106**:3028-3034.

Nathan, C. (2006). Neutrophils and immunity: Challenges and opportunities. *Nature Reviews Immunology*, **6**:173–82.

Neer, T.M. (1988). Clinical pharmacologic features of fluoroquinolone antimicrobial drugs. *Journal of the American Veterinary Medical Association*, **193**:577–580.

Nelson, R. F. (2005). An introduction to behavioral endocrinology (3rd ed.). Sunderland, Mass: Sinauer Associates. p. 100. ISBN 0-87893-617-3.

Neu, H.C. (1988). Quinolones: A new class of antimicrobial agents with wide potential uses. *Medical Clinics of North America,* **72**:623–636.

Newton, P.M., Watson, J.A., Wolowacz, R.G. and Wood, E.J. (2004). Macrophages Restrain Contraction of an In Vitro Wound Healing Model. *Inflammation*, **28**(4): 207–14.

Nicklin, M.J., Weith, A. and Duff, G.W. (1994). A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta, and interleukin-1 receptor antagonist genes. *Genomics*, **19**(2): 382–4.

Niiya, K., Hodson, E., Bader, R., Byers-Ward, V., Koziol, J.A., Plow, E.F. and Ruggeri, Z.M. (1987). Increased surface expression of the membrane glycoprotein IIb/IIIa complex induced by platelet activation. Relationship to the binding of fibrinogen and platelet aggregation. *Blood,* **70**:475-483.

Noszal, B., Kraszni, M., Racz, A. (2004). Histamine: fundamentals of biological chemistry. In Falus, A., Grosman, N. and Darvas, Z. Histamine: Biology and Medical Aspects. Budapest: SpringMed. pp. 15–28.

Ovchinnikov, D.A. (2008). Macrophages in the embryo and beyond: Much more than just giant phagocytes. *Institute for Molecular Bioscience and Cooperative Research Centre for Chronic Inflammatory Diseases (CRC-CID),* **46**(9): 447–62.

Pankey, G. A. and Sabath, L. D. (2004). Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clinical Infectious Diseases,* **38** (6): 864–870.

Panula, P., Chazot, P.L. and Cowart, M. (2015). International Union of Basic and Clinical Pharmacology. XCVIII. Histamine Receptors. *Pharmacological Reviews*, **67**(3): 601–55.

Parakrama, C. and Clive, R. T. (2005). Part A. General Pathology, Section II. The Host Response to Injury, Chapter 3. The Acute Inflammatory Response, sub-section Cardinal Clinical Signs. Concise Pathology (3rd Ed.). New York, N.Y.: McGraw-Hill. ISBN 0-8385-1499-5.

Parkhouse, J. and Pleuvry, B. J. (1979). Analgesic drugs. Blackwell Co., Oxford. p. 1.

Parpura, V., Basarsky, T.A., Liu, F., Jeftinija, K., Jeftinija, S. and Haydon, P.G. (1994). Glutamate-mediated astrocyte-neuron signalling. *Nature,* **369**(6483): 744–7.

Pecora, P. G. and Kaplan, B. (1996). Corticosteroids and ulcers: is there an association? *Annals of Pharmacotherapy*, **30**(7-8): 870-872.

Peitsch, M.C. and Tschopp, J. (1991). Assembly of macromolecular pores by immune defence systems. *Current Opinion in Cell Biology*, **3**(4): 710–716.

Piddock, L.J. (1994). New quinolones and Gram-positive bacteria. *Antimicrobial Agents and Chemotherapy,* **38**:163–169.

Plewig, G. and Schopf, E. (1976). Anti-inflammatory effects of antimicrobial agents. *Drugs*, **11**:472–473.

Polyzoidis, S., Koletsa, T., Panagiotidou, S., Ashkan, K. and Theoharides, T. C. (2015). Mast cells in meningiomas and brain inflammation. *Journal of Neuroinflammation*, **12**(1): 170.

Porth, C. (2007). Essentials of pahtophysiology: concepts of altered health states. Lippincott Williams & Wilkins. p. 270. ISBN 0-7817-7087-4.

Preheim, L., Cuevas, T., Roccaforte, J., Mellencamp, M. and Bittner M. (1987). Oral ciprofloxacin in the treatment of elderly patients with complicated urinary tract infections due to trimethoprim/sulfamethoxazole-resistant bacteria. *The American Journal of Medicine*, **82**:295–297.

Prussin, C. and Metcalfe, D. D. (2003). 4. IgE, mast cells, basophils, and eosinophils. *The Journal of Allergy and Clinical Immunology*. **111**(2): 486-494.

Rand, M.L., Leung, R. and Packham, M.A. Platelet function assays. *Transfusion and Apheresis Sciences,* **28**:307-317.

Rather, L. J. (1971). Disturbance of function (functio laesa): the legendary fifth cardinal sign of inflammation, added by Galen to the four cardinal signs of Celsus. *Bulletin of the New York Academy of Medicine,* **47**(3): 303–322.

Reeves, E.P., Williamson, M., O'Neill, S.J., Greally, P. and McElvaney, N.G. (2011). Nebulized hypertonic saline decreases IL-8 in sputum of patients with cystic fibrosis. *American Journal of Respiratory and Critical Care Medicine*, **183**(11): 1517–23.

Rhee, K. Y. and Gardiner, D. F. (2004). Clinical relevance of bacteriostatic versus bactericidal activity in the treatment of gram-positive bacterial infections. *Clinical Infectious Disease,* **39**(5): 755–6.

Roberts, J. L. and Morrow, J. D. (2001). Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout In: (eds Gilman, A. G., Hardman, J. G., Limbird, L. E.) 10th Edn. Goodnam and Gilman,s the pharmacological basis of therapeutics. McGraw Hill Co., New York, pp. 687-731.

Royle, N.J., Irwin, D.M., Koschinsky, M.L., MacGillivray, R.T. and Hamerton, J.L. (1987). Human genes encoding prothrombin and ceruloplasmin map to 11p11-11q12 and 3q21-3q24 respectively. *Somatic Cell and Molecular Genetics*, **13**(3): 285–92.

Sahu, A. and Lambris, J.D. (2001). Structure and biology of complement protein C3, a connecting link between innate and acquired immunity. *Immunological Reviews,* **180**:35–48.

Sárközy, G. (2001). Quinolones: a class of antimicrobial agents. *Veterinary Medicine Czech*, **46**(9-10): 257-274.

Schimmer, B. P. and Parker, K. L. (2001). Adrenocorticotrophic hormone; adrenocortical steroids and their synthetic analogues; inhibitors of the synthesis and actions of adrenocortical hormones In: (Gilman, A. G., Hardman, J. G., Limbird, L. E. Eds) 10th Ed. Goodman and Gilman's the pharmacological basis of therapeutics. McGraw Hill Co., New York, p. 1649-1677.

Schmitt, A., Hauser, C., Jaunin, F., Dayer, J.M. and Saurat, J.H. (1986). Normal epidermis contains high amounts of natural tissue IL 1 biochemical analysis by HPLC identifies a MW approximately 17 Kd form with a P1 5.7 and a MW approximately 30 Kd form. *Lymphokine Research*, **5**(2): 105–18.

Schoenborn, J.R. and Wilson, C.B. (2007). Regulation of interferon-gamma during innate and adaptive immune responses. *Advances in Immunology*, **96**: 41–101.

Schroder, K., Hertzog, P.J., Ravasi, T. and Hume, D.A. (2004). Interferon-gamma: an overview of signals, mechanisms and functions. *Journal of Leukocyte Biology*, **75**(2): 163–89.

Sharma, K. K., Khanna, T. and Sen, P. (1982). Current status of centrally acting peptides. In: Dhawan, B. N., editor. Advances in Bioscience. Vol. 38. Pergamon Press, Oxford. p. 147

Shattil, S.J. and Newman, P.J. (2004). Integrins: dynamic scaffolds for adhesion and signalling in platelets. *Blood*, **104**:1606-1615.

Shaw, S. K., Bamba, P. S., Perkins, B. N. and Luscinskas, F. W. (2001). Real-time imaging of vascular endothelial-cadherin during leukocyte transmigration across endothelium. *Journal of Immunology*, **167**:2323-30.

Shiri, R., Koskimäki, J., Häkkinen, J., Tammela, T. L., Auvinen, A. and Hakama, M. (2006). Effect of non-steroidal anti-inflammatory drug use on the incidence of erectile dysfunction. *Journal of Urology*, 175(5)

Smith J.T. (1986). The mode of action of 4-quinolones and possible mechanisms of resistance. *Journal of Antimicrobial Chemotherapy,* **18**:21–29.

Sobocka, M.B., Sobocki, T., Babinska, A., Hartwig, J.H., Li, M., Ehrlich, Y.H. and Kornecki, E. (2004). Signalling pathways of the F11 receptor (F11R; a.k.a. JAM-1, JAM-A) in human platelets: F11R dimerization, phosphorylation and complex formation with the integrin GPIIIa. *Journal of Receptors and Signal Transduction Research*, **24**:85-105.

Stanley, K.K., Marazziti, D., Eggertsen, G. and Fey, G.H. (1988). Relationships between the gene and protein structure in human complement component C9. *Biochemistry*, **27**(17): 6529–6534.

Stashak, T.S., Farstvedt, E. and Othic, A. (2004). Update on wound dressings: Indications and best use. *Clinical Techniques in Equine Practice,* **3**(2): 148–163.

Stryer, Lubert. (1995). Biochemistry, 4th Edition. W.H. Freeman and Company. p. 732.

Swirski, F.K., Nahrendorf, M., Etzrodt, M., Wildgruber, M., Cortez-Retamozo, V., Panizzi, P., Figueiredo, J.L., Kohler, R.H., Chudnovskiy, A., Waterman, P., Aikawa, E., Mempel, T.R., Libby, P., Weissleder, R. and Pittet, M.J. (2009). Identification of Splenic Reservoir Monocytes and Their Deployment to Inflammatory Sites. *Science*, **325**(5940): 612–616.

Tak, T., Tesselaar, K., Pillay, J., Borghans, J.A. and Koenderman, L. (2013). What's your age again? Determination of human neutrophil half-lives revisited. *Journal of Leukocyte Biology*, **94**(4): 595–601.

Takeshita, K., Yamagishi, I., Harada, M., Otomo, S., Nakagawa, T. and Mizushima, Y. (1989). Immunological and anti-inflammatory effects of clarithromycin: Inhibition of interleukin 1 production of murine peritoneal macrophages. *Drugs under Experimental and Clinical Research*, **15**:527–533.

Tarayre, J.P., Aliaga, M., Barbara, M., Villanova, G., Ballester, R., Tisne-Versailles, J. and Couzinier, J.P. (1987). Cutaneously applied erythromycin base reduces various types of inflammatory reactions in mouse ear. *International Journal of Tissue Reactions,* **9**:77–85.

Tenson, T., Lovmar, M. and Ehrenberg, M. (2003). The Mechanism of Action of Macrolides, Lincosamides and Streptogramin B Reveals the Nascent Peptide Exit Path in the Ribosome. *Journal of Molecular Biology*, **330** (5):1005–1014.

Traversa, G., Walker, A. M., Ippolito, F. M., Caffari, B., Capurso, L., Dezi, A, Koch, M., Maggini, M., Alegiani, S. S. and Raschetti, R. (1995). Gastroduodenal toxicity of different nonsteroidal anti-inflammatory drugs. *Epidemiology*, **6**(1): 49-54.

Trelle, S., Reichenbach, S., Wandel, S., Hildebrand, P., Tschannen, B., Villiger, P. M., Egger, M. and Jüni, P. (2011). Cardiovascular safety of non-steroidal anti-inflammatory drugs: network meta-analysis. *BMJ*, **342**.

Utgaard, J.O., Jahnsen, F.L., Bakka, A., Brandtzaeg, P. and Haraldsen, G. (1998). Rapid secretion of prestored interleukin-8 from Weibel-Palade bodies of microvascular endothelial cells. *Journal of Experimental Medicine,* **188**(9): 1751–6.

Varga-Szabo, D., Pleines, I. and Nieswandt, B. (2008). Cell adhesion mechanisms in platelets. *Arteriosclerosis, Thrombosis and Vascular Biology*, **28**: 403-412.

Varga-Szabo, D., Braun, A. and Nieswandt, B. (2009). Calcium signalling in platelets. *Journal of Thrombosis and Haemostasis,* **7**:1057-1066.

Vega, M. A. and Corbi, A. L. (2006). Human macrophage activation: Too many functions and phenotypes for a single cell type. *Immunologia,* **25**:248-272.

Voehringer, D. (2009). The role of basophils in helminth infection. *Trends in Parasitology*, **25**(12): 551–6.

Vogel, W. H. and Berke, A. (2009). Brief History of Vision and Ocular Medicine. Kugler Publications. p. 97. ISBN 90-6299-220-X?

Voight, W.H. (1987). Electron microscopic studies on the effect of a quinolone carboxylic acid derivative on the ultrastructure of Escherichia coli and staphylococcal bacteria in vitro. *Veterinary Medicine Reviews,* **2**:119–121.

Wallace, J. M. (2002). Nutritional and botanical modulation of the inflammatory cascade: eicosanoids, cxyclooxgenase and lipooxygenase-as an adjunct in cancer therapy. *Integrative Cancer Therapies*, **1**: 7-37.

Ward, P.A. (2004). The dark side of C5a in sepsis. *Nature Reviews Immunology,* **4**(2): 133–42.

Waugh, D.J. and Wilson, C. (2008). The interleukin-8 pathway in cancer. *Clinical Cancer Research,* **14**(21): 6735–41.

Wiegand, I., Hilpert, K. and Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols,* **3**(2): 163–175.

Winter, C. A., Risley, E. A. and Nuss, G. W. (1962). Carrageenin-induced oedema in the hind paw of a rat as an assay for anti-inflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine*, **111**:544-547.

Witko-Sarsat, V., Rieu, P., Descamps-Latscha, B., Lesavre, P. and Halbwachs-Mecarelli, L. (2000). Neutrophils: molecules, functions and pathophysiological aspects. *Laboratory Investigation*, **80**(5): 617–53.

Wolff, B., Burns, A.R., Middleton, J. and Rot, A. (1998). Endothelial cell "memory" of inflammatory stimulation: human venular endothelial cells store interleukin 8 in Weibel-Palade bodies. *The Journal of Experimental Medicine*, **188**(9): 1757–62.

Yadav, R., Karen, Y.L., Rebecca, E.Y. and Sussan, N. (2003). Migration of leukocytes through the vessel wall and beyond. *Journal of Thrombosis and Haemostasis*, **90**:598–606

Yankanchi, S. R. and Koli, S. A. (2010). Anti-inflammatory and analgesic activity of mature leaves and methanol extract of *Clerodendrum inerme* L. (Gaertn). *Journal of Pharmaceutical Sciences and Research*, **11**(2): 782-785.

Young, B., Lowe, J.S., Stevens, A. and Heath, J.W. (2006). Wheater's Functional Histology (5 Ed.). Elsevier Limited. ISBN 0-443-06850.

Zucker-Franklin, D., Greaves, M.F., Grossi, C. E. and Marmont, A.M. (1988). Neutrophils. Atlas of Blood Cells: Function and Pathology 1 (2nd Ed.). Philadelphia: Lea & Ferbiger.