University of Nigeria

Virtual Library

Serial No.	
Author 1	ONWUKA, NGOZI AMANDA (PG/M.PHARM/09/51479)
Author 2	
Author 3	
Title:	EVALUATION OF THE IMMUNOMODULATORY ACTIVITY OF *HUSLUNDIA OPPOSITA* VAHL
Keyword:	
Description:	DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY
Category:	FACULTY OF PHARMACEUTICAL SCIENCES
Publisher:	
Publication Date:	
Signature:	Ogbonna Nkiru Digitally Signed by: Content manager's Name DN : CN = Webmaster's name O= University of Nigeria, Nsukka OU = Innovation Centre

EVALUATION OF THE IMMUNOMODULATORY ACTIVITY

of *Hoslundia opposita* Vahl (Lamiaceae) Leaf Extract

BY

ONWUKA, NGOZI AMANDA

(PG/M.PHARM/09/51479)

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

FACULTY OF PHARMACEUTICAL SCIENCES

UNIVERSITY OF NIGERIA, NSUKKA

JULY, 2012

EVALUATION OF THE IMMUNOMODULATORY ACTIVITY of *Hoslundia opposita* Vahl (Lamiaceae) Leaf Extract

BY
ONWUKA, NGOZI AMANDA
(PG/M.PHARM/09/51479)

A PROJECT REPORT PRESENTED TO
THE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY
FACULTY OF PHARMACEUTICAL SCIENCES
UNIVERSITY OF NIGERIA, NSUKKA
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF
MASTER OF PHARMACY
(M. PHARM) DEGREE.

DR A.C. EZIKE & PHARM O.O. NDU (SUPERVISORS)
DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY
FACULTY OF PHARMACEUTICAL SCIENCES
UNIVERSITY OF NIGERIA, NSUKKA

JULY, 2012

TITLE PAGE

EVALUATION OF THE IMMUNOMODULATORY ACTIVITY of *Hoslundia opposita* Vahl (Lamiaceae) Leaf Extract

CERTIFICATION

ONWUKA, NGOZI AMANDA, a postgraduate student of the Department of Pharmacology and Toxicology with Registration Number **PG/M.Pharm/09/51479,** has satisfactorily completed the requirements for the award of the degree of Master of Pharmacy (M.Pharm) of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

The work embodied in this project is original and has not been submitted in part or full for any other diploma or degree of this or any other university.

ONWUKA, N.A.

Student

DR. A.C. EZIKE

Supervisor

PHARM
O.O. NDU

Supervisor

PROF. C.O. OKOLI

Head of Department

DEDICATION

This work is dedicated to my daughter, Exalted and her siblings who thrive for excellence in life. I love you all.

ACKNOWLEGDEMENTS

I cannot appreciate God, Divine intelligence, enough. When all hope was lost, He stepped into my life, gave me a new beginning and made my life beautiful. For Him, I live. God, I love you.

I am highly indebted to my supervisors. Dr A.C. Ezike did not only supervise me, she was and remains a mother to me. She gave me emotional support when I almost gave up. She also provided financially. My God will answer her when money cannot. Pharm O.O. Ndu is a father. May God bless him.

My parents, Mr. & Mrs. O.M. Onwuka, are instruments of honour. They put me on the path of excellence. My grandmother, Mrs. Okorie, gave me an invaluable support. She was my baby's nanny while on this programme. She shall reap the fruits of her labour.

To my lover-in-chief and sweetheart, Dr N.N. Nwankpa, thank you for standing by me even when there were reasons to walk out. Your love has stood the test of time; because you are here, I concluded this work with great enthusiasm. You have been a husband, father, lover, adviser, and companion to me. May Jesus ever stand by you. Thanks to the gift of love, our daughter, Exalted, who gave me maximum support by allowing me do this work.

Thanks to my siblings: Mrs Chinyere Igwe, Mrs Gift Chukwuma, Bishop Onwuka Uchechukwu and Rev. Onwuka Chizurum. Immeasurable thanks to my little

Angel Kodi and Sweet Fairer, Oleka Samuel, who worked late into the night with me. You are great.

Huge thanks to Chief & Barr. (Mrs.) Cornel Umeh; they were the brain behind the concept of this work and also provided the plant used for preliminary studies. May God keep you.

I thank the Chaplain of Christ Church Chapel, Venerable B.N. Ezeh, who made me start this postgraduate programme.

I am grateful to Dr. Jayeola of the University of Agriculture, Abeokuta. He was instrumental in collecting and cleaning the plant from Aregbe, Obantoko, Abeokuta in Ogun State. May God bless you and your family. Much thanks to Brother Osasona Sola who was also instrumental in collecting, cleaning and taking the plant to the Forestry Research Institute of Nigeria (FRIN) for voucher specimen number. Your purpose on earth will be established.

Thanks to Prof. Charles Okoli, Head of Department, Department of Pharmacology and Toxicology, UNN. I thought he was a difficult, unapproachable man, but by his persistence, care, and concern, I continued. May God establish him.

I am very grateful to Mr. Sebastin Igboeme of the Department of Clinical Pharmacy & Pharmacy Management. He was always willing to assist even into the nights without anticipating rewards. My God will reward him. I am also thankful to Pharm I.A. Nwabunike. She gave me great support from her wealth of knowledge. God bless you.

I appreciate Mr. Emmanuel Ezichi of the Medical Laboratory Department, Medical Center, University of Nigeria, Nsukka (UNN). I am thankful to Dr. Idika and Oga Austin, both of Faculty of Veterinary Medicine, UNN. They were available even at the slightest notice.

I appreciate the entire staff of the Dept. of Pharmacology and Toxicology, UNN, especially Prof. P.A. Akah, Dr. C.S. Nworu, Mr. Bonny Ezeh, Mrs Ego, Mrs Ugwu and Dr. Michelle of InterCEDD, Nsukka for their timely suggestion, assistance and encouragement.

ABSTRACT

The immunomodulatory properties of methanol extract and fractions of *Hoslundia opposita* leaf on Delayed-Type Hypersensitivity (DTH), primary and secondary humoral response and *in vivo* leucocyte mobilization were evaluated. Acute toxicity test of crude extract and phytochemistry study was carried out. The methanol extract (ME) at 200, 400 and 800 mg/kg body weight produced significant ($P<0.05$) inhibition of DTH response in rat by 85, 75 and 85%. The n-hexane, ethyl acetate and methanol fractions at 200, 400 and 800 mg/kg body weight produced significant ($P<0.05$) inhibition of DTH response. Treatment of rats with single intraperitoneal injection of carrageenan after oral administration of extract and fractions resulted in an increase in leucocyte mobilization into the rat's peritoneal fluid which was significant. The total leucocytes counts (TLC) were higher in the extract and fractions treated groups when compared to the control group. However a fall in primary and secondary antibody titre was observed with both ME and fractions suggesting that *Hoslundia opposita* may act through a cell mediated mechanism. The crude extract administered (orally) at 5000 mg/kg did not cause lethality after 48 h observation period. Phytochemistry tests revealed abundant presence of resins, terpenoids, flavonoids, saponins and glycosides in the fractions and crude extract. These findings suggest that these may be responsible for the immunomodulatory effect of the leaves of *Hoslundia opposita*. It was therefore recommended that further studies be done to identify and isolate the exact constituent responsible for the immunomodulatory effects and also establish mechanism of action of *Hoslundia opposita* Vahl (Lamiaceae).

TABLE OF CONTENTS

Chapter Two: Materials and method

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 Introduction

1.1.1 History of Immunology

Immunology is a broad branch of biomedical science that covers the study of all aspects of immune system in all living organisms. It is the branch of medicine and biology concerned with immunity. Immunity is a biological term that describes a state of having sufficient biological defenses to avoid infection, disease, or other unwanted biological invasion. The concept of immunity has intrigued mankind for thousands of years (Silverstein, 1989). The prehistoric view of disease was that it was caused by supernatural forces, and that illness was a form of theurgic punishment for “bad deeds” or “evil thoughts” visited upon the soul by the gods or by one’s enemies (Lindquester, 2006). The ancient historic view was that disease was spontaneously generated instead of being created by microorganisms that grow by reproduction (Madigan and Martinko, 2005). Between the time of Hippocrates and the 19th century, when the foundations of the scientific methods were laid, diseases were attributed to an alteration or imbalance in one of the four humors (blood, phlegm, yellow bile or black bile), (Silverstein, 1989). Also popular during this time was the miasma theory, which held that diseases such as cholera or the Black Plague were caused by a miasma, a noxious form of "bad air" (Lindquester, 2006). If someone were exposed to the miasma, they could get the disease.

The word “immunity” derives from the Latin word: *immunis*, meaning exemption from military service, tax payments or other public services (Gherardi, 2006). The

first written descriptions of the concept of immunity may have been made by the Athenian, Thucydides who, in 430 BC, described that when the plague hit Athens *"*the sick and the dying were tended by the pitying care of those who had recovered, because they knew the course of the disease and were themselves free from apprehensions. For no one was ever attacked a second time, or not with a fatal result*"* (Gherardi, 2006). The term "immunes", is also found in the epic poem "Pharsalia" written around 60 B.C. by the poet Marcus Annaeus Lucanus to describe a North African tribe's resistance to snake venom (Silverstein, 1989).

The first clinical description of immunity which arose from a specific disease causing organism is probably *Kitab fi al-jadari wa-al-hasbah* (*A Treatise on Smallpox and Measles*), translated in 1848 (Al-Razi, 2003) and written by the Islamic physician, Al-Razi in the 9th century. In the treatise, Al-Razi describes the clinical presentation of smallpox and measles and goes on to indicate that exposure to these specific agents confers lasting immunity (although he did not use this term) (Silverstein, 1989). However, it was with Louis Pasteur's Germ theory of disease, which states that many diseases are caused by the presence and actions of specific micro-organisms within the body (Worboys, 2008), that the fledgling science of immunology began to explain how bacteria caused disease, and how, following infection, the human body gained the ability to resist further infections (Gherardi, 2006), though microorganisms were first directly observed by Anton Van Leeuwenhoek, who is considered the father of microbiology. Building on Leeuwenhoek's work, Nicolas Andry argued in 1700 that microorganisms (which he called worms) were responsible for smallpox and other diseases (Andry, 1700).

1.1.1.1 The role of smallpox in the development of vaccination

Immunology is the study of the immune system, which protects organisms against disease, while a vaccine is an agent that helps make the body immune to a specific disease or illness. The vaccine triggers the immune system's infection-fighting ability and memory without exposure to the actual disease-producing germs. The immunity developed following vaccination is similar to the immunity acquired from natural infection.

Immunization had existed in various forms for at least a thousand years, (Gherardi, 2006). The earliest use of immunization is unknown, but around 1000 AD, the Chinese began practicing a form of immunization by drying and inhaling powders derived from the crusts of smallpox lesions (Gherardi, 2006). However, the earliest recognized attempt to intentionally induce immunity to an infectious disease was in the 10th century in China, where smallpox was endemic. The process of "variolation" involved exposing healthy people to material from the lesions caused by the disease, either by putting it under the skin, or, more often, inserting powdered scabs from smallpox pustules into the nose. Variolation was known and practiced frequently in the Ottoman Empire, where it had been introduced by Circassian traders around 1670, (Gherardi, 2006). Unfortunately, because there was no standardization of the inoculum, the variolation occasionally resulted in death or disfigurement from smallpox, thus limiting its acceptance.

Variolation later became popular in England, mainly due to the efforts of Lady Mary Wortley Montague (Gherardi, 2006) who survived smallpox but lost a brother to it. Lady Montague was married to Lord Edward Wortley Montague, the

ambassador to the Sublime Porte of the Ottomans in Istanbul. While in Istanbul, Lady Montague observed the practice of variolation. Determined not to have her family suffer as she had, she directed the surgeon of the Embassy to learn the technique and, in March 1718, to variolate her five year-old son. After her return to England, she promoted the technique, and had her surgeon variolate her four year-old daughter in the presence of the king's physician (Greenberg, 1957). The surgeon, Charles Maitland, was given leave to perform what came to be known as the Royal Experiment, in which he variolated six condemned prisoners who later survived. By these and other experiments, the safety of the procedure was established. Subsequently, the practice of variolation spread rapidly throughout England in the 1740s and then to the American colonies.

1.1.1.2 Edward Jenner and the development of the first safe vaccine for smallpox

Although Jenner is celebrated for his development of cowpox as a safe vaccine for smallpox, he was not the first to make use of a relatively non-pathogenic virus to induce

immunity. In 1774, Benjamin Jesty, a farmer, inoculated his wife with the vaccinia virus

obtained from "farmer Elford of Chittenhall, near Yetminster." In 1796, Jenner inoculated James Phipps with material obtained from a cowpox lesion that appeared on the hand of a dairymaid. (Silverstein, 1989). Six weeks later, he inoculated the experimental subject with smallpox without producing disease. Although this experiment justifiably lacked an appropriate control, further studies by Jenner

established the efficacy of his vaccination procedure (Jerner, 1955). For this feat, Jenner received a cash prize of 30,000 pounds and election to nearly all of the learned societies throughout Europe (Silverstein, 1989).

1.1.1.3 Koch, Pasteur, and the germ theory of disease

In 1875, Robert Koch, a country physician with no formal scientific training, inoculated the ear of a rabbit with the blood of an animal that had died of anthrax. The rabbit died the next day. He isolated infected lymph nodes from the rabbit and was able to show that the bacteria contained within them could transfer disease to other animals. He developed and refined techniques necessary for the cultivation of bacteria, including the development of agar growth medium (Greenberg, 1957). He was appointed to the Institute of Hygiene in Berlin, where his ultimate goal was to identify the organism responsible for the "White Death"-tuberculosis.

Quite independently, Louis Pasteur began his studies of the "chicken cholera bacillus." In a serendipitous discovery, Pasteur inadvertently left a flask of the bacillus on the bench over the summer and inoculated 8 chickens with this "old but viable" stock of chicken cholera bacillus (Greenberg, 1957). He found that not only did the chickens not die, but they did not even appear ill! Pasteur said that the virulent chicken cholera bacillus had become attenuated by sitting on the bench over the summer months. The similarity between these results and those of Jenner using vaccinia virus was immediately apparent to him. In honor of Jenner, Pasteur called his treatment vaccination. Pasteur later worked on anthrax and rabies and developed the first viable vaccine for anthrax and rabies.

Although Koch and Pasteur were contemporaries, they were intensely competitive and actually bitter enemies; of course, the outbreak of the Franco-Prussian war (1870) did nothing to cement their relationship (Greenberg, 1957). In a trenchant example of how not to behave toward a colleague at a scientific meeting, Koch made his way to the podium following Pasteur's lecture and said: "When I saw in the program that Monsieur Pasteur was to speak today...I attended the meeting eagerly, hoping to learn something new...I must confess that I have been disappointed, as there is nothing new in the speech which Monsieur Pasteur has just made..."

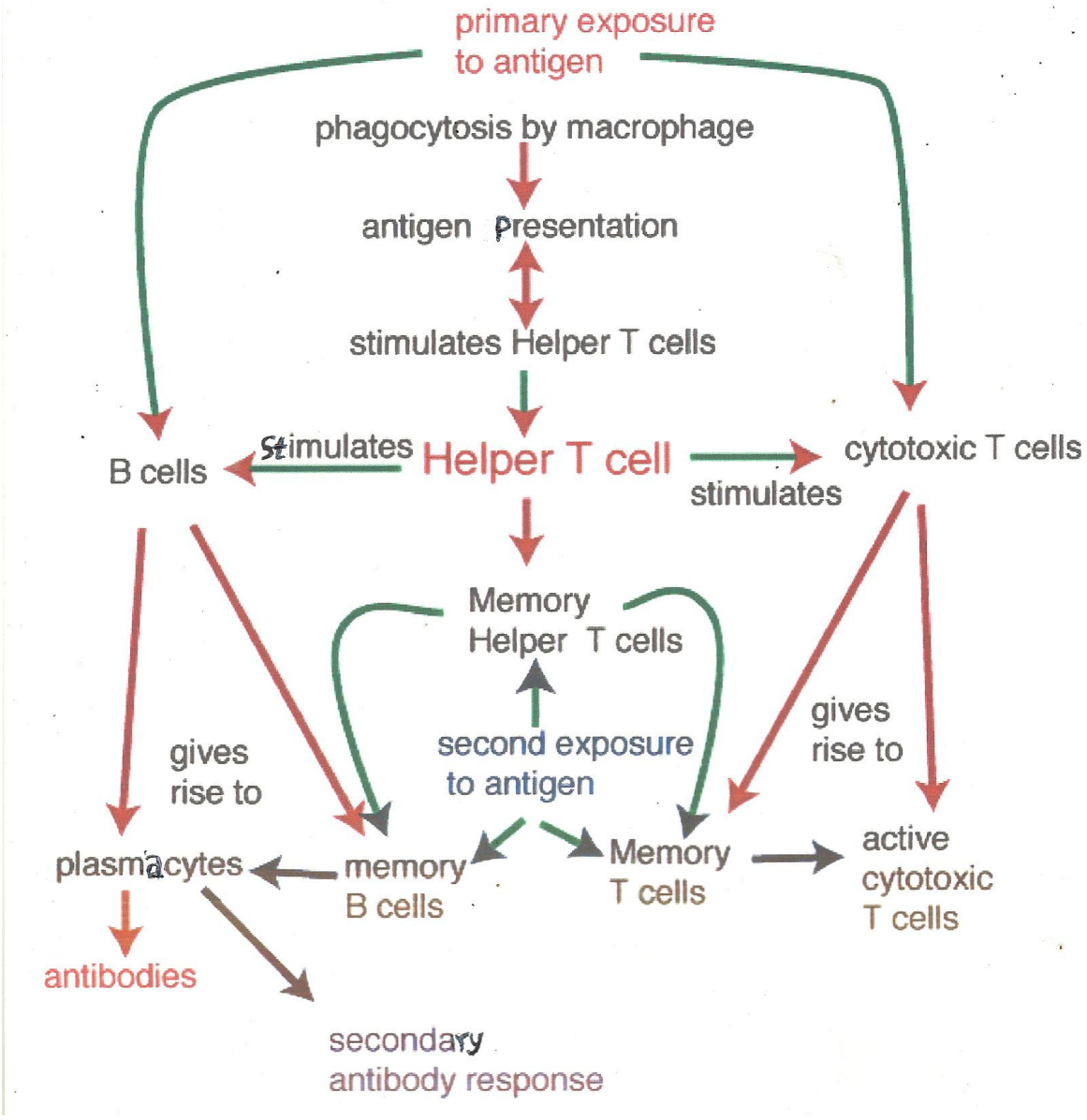
Although many consider Pasteur the "father of immunology" both his and Koch's efforts firmly established the germ theory of disease. Prior to this time, although the practical benefits of variolation were apparent, there was no known biological basis for either the cause of diseases or the efficacy of vaccination.

1.1.2 Immune response

The immune response is how the body recognizes and defends itself against bacteria, viruses, and substances that appear foreign and harmful to the body (Fig.1). The immune system protects the body from potentially harmful substances by recognizing and responding to antigens (Firestein, 2007). Antigen is any molecule that can interact with antibodies whereas immunogen is any molecule that induces or elicits an immune response. All immunogens are antigens but all antigens may not be immunogenic e.g. hapten. Therefore, hapten is an antigen that

is not immunogenic by itself. Antigens include molecules (usually proteins) on the surface of cells, viruses, fungi, or bacteria; and also non-living substances such as toxins, chemicals, drugs, and foreign particles (such as a splinter). The immune system recognizes and destroys substances that contain these antigens (Goronzy and Weyand, 2007). Even the human body cells have proteins that are antigens. These include a group of antigens called human leukocyte antigens (HLA). The immune system learns to see these antigens as normal and does not usually react against them.

Figure 1: Immune response



(Source: *The New York Times*, 12/1/2012, Immune response)

The types of immune responses are:

(a) Innate/ Non-specific immunity which is the defense system a person has at birth. It protects one against all antigens. Innate immunity involves barriers that keep harmful materials from entering the body. These barriers form the first line of defense in the immune response.

(b) Acquired / Adaptive immunity is immunity that develops with exposure to various antigens. The immune system builds a defense that is specific to that antigen (Alan et al., 2001).

(c) Passive immunity is due to antibodies that are produced in a body other than one's own. Infants have passive immunity because they are born with antibodies that are transferred through the placenta from their mother. These antibodies disappear between 6 and 12 months of age.

1.1.2.1 Innate/ Non-specific immunity

The innate immune response has usually been rather airily dismissed by host immunologists as being an ancient throwback that merely provides a temporary holding operation until the more effective specific adaptive immune response gets going (Rang et al, 2007). Rather, the innate response has a much more significant role in host defence. The innate immune system is the body's first line of defense against invading organisms. Microorganisms or toxins that successfully enter an organism will encounter the cells and mechanisms of the innate immune system. The innate response is usually triggered when microbes are identified by pattern

recognition receptors, which recognize components that are conserved among broad groups of microorganisms,http://en.wikipedia.org/wiki/Immune_system_-_cite_note-pmid17943118-25 or when damaged, injured or stressed cells send out alarm signals, many of which (but not all) are recognized by the same receptors as those that recognize pathogens. Innate immune defenses are non-specific, meaning these systems respond to pathogens in a generic way. This system does not confer long-lasting immunity against a pathogen. The innate immune system is the dominant system of host defense in most organisms (Goronzy and Weyand, 2007).

1.1.2.1.1 Components of the Innate Immune System

1.1.2.1.1.1 Anatomic Barriers

The first line of defense of the body is the skin and other anatomic barriers to invasion. These include tears, saliva, mucus and cilia in the intestinal and respiratory tracts (Goronzy and Weyand, 2007). The factors responsible for anatomic barriers are:

1. Mechanical factors

The epithelial surfaces form a physical barrier that is very impermeable to most infectious agents. Thus, the skin acts as our first line of defense against invading organisms. The desquamation of skin epithelium also helps remove bacteria and other infectious agents that have adhered to the epithelial surfaces. Movement due to cilia or peristalsis helps to keep air passages and the gastrointestinal tract free from microorganisms (Male et al., 2006). The flushing action of tears and saliva helps prevent infection of the eyes and mouth. The trapping effect of mucus that lines the respiratory and gastrointestinal tract helps protect the lungs and digestive systems from infection (Male et al., 2006). Effector mechanisms of

these factors are found in table 1.

2. Chemical factors

Fatty acids in sweat inhibit the growth of bacteria. Lysozyme and phospholipase found in tears, saliva and nasal secretions (table 1), can breakdown the cell wall of bacteria and destabilize bacterial membranes. The low pH of sweat and gastric secretions prevents growth of bacteria (Male et al., 2006). Defensins (low molecular weight proteins) found in the lung and gastrointestinal tract have antimicrobial activity. Surfactants in the lung act as

opsonins (substances that promote phagocytosis of particles by phagocytic cells) (Kendall, 2007).

3. Biological factors

The normal flora of the skin and the gastrointestinal tract can prevent the colonization of pathogenic bacteria by secreting toxic substances or by competing with pathogenic bacteria for nutrients or attachment to cell surfaces (Gene, 2011).

1.1.2.1.1.2 Humoral and chemical barrier

The anatomical barriers are very effective in preventing colonization of tissues by microorganisms. However, when there is damage to tissues, the anatomical barriers are breached and infection may occur (Goronzy and Weyand, 2007). Once infectious agents have penetrated tissues, another innate defense mechanism comes into play, namely acute inflammation. Humoral factors play an important role in inflammation, which is characterized by edema and the recruitment of phagocytic

cells (Goronzy and Weyand, 2007). These humoral factors are found in serum or they are formed at the site of infection.

1.1.2.1.1.2.1 Inflammation

The inflammatory response occurs when tissues are injured by bacteria, trauma, toxins, heat or any other cause. The damaged cells release chemicals including histamine, bradykinin, and prostaglandins. These chemicals cause blood vessels to leak fluid into the tissue causing swelling which helps isolate the foreign substance from further contact with body tissues (Firestein, 2007). The local manifestations of acute reaction to an invading organism are redness, swelling, heat and pain as the cardinal signs of inflammation; a fifth is loss of function (Rang et al., 2007). In addition, to the local changes in an inflammatory area, there are often general systemic manifestations of inflammatory disease including fever, leucocytosis and the release from the liver of acute phase proteins e.g. C-reactive protein, α2-macroglobulin, fibrinogen, α1-antitrypsin and some complement components. The C reactive proteins bind to some micro-organisms and the resulting complex activates complement systems. Other proteins scavenge iron (an essential nutrient for invading organisms) or block proteases perhaps protecting the host against the worst excesses of an inflammatory response (Rang et al., 2011). Cortisol also increases and exerts an important counter-regulatory effect and inflammatory response. Mast cell mediators of inflammatory processes are histamine, proteases, heparin, leukotriene C_4 and B_4, prostaglandin D_2, platelet activating factor, major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin,

interleukin-1 and 6, granulocyte/macrophage colony stimulating factor and tumor necrosis factor (Undem and Lichtenstein, 2001).

Inflammatory response is a defence mechanism and not a disease (Rang et al., 2007). Its role is to restore normal structure and function to the infected/damaged tissue and in the vast majority of cases, this is what happens. The healing and resolution phase of the inflammatory response is an active process that utilizes its own unique palette of mediators and cytokines to terminate residual inflammation and to promote remodeling and repair of damaged tissue. Infectious diseases such as syphilis, tuberculosis and leprosy bear the characteristic hallmark of chronic inflammation from the start (Rang et al., 2007). The cellular and mediator components of this type of inflammation are also seen in many if not most chronic autoimmune and hypersensitivity disease and are important targets for drug action.

1.1.2.1.1.2.2 Complement System and others

1. Complement system – Complement was discovered by Jules Bordet (1870-1961) (Hunt and Mitzi, 2004). Historically, the term complement (C) was used to refer to a heat-labile serum component that was able to lyse bacteria; its activity is destroyed (inactivated) by heating serum at 56°C for 30 minutes. However, complement is now known to contribute to host defenses in other ways as well. Complement can opsonize bacteria for enhanced phagocytosis; it can recruit and activate various cells including polymorphonuclear cells (PMNs) and macrophages; it can participate in regulation of antibody responses and it can aid in the clearance of immune complexes and apoptotic cells. Complement can also have detrimental effects for the host; it contributes to inflammation and tissue damage and it can

trigger anaphylaxis. The complement system is the major humoral non-specific defense mechanism.

Complement comprises over 20 different serum proteins that are produced by a variety of cells including, hepatocytes, macrophages and gut epithelial cells. Some complement proteins bind to immunoglobulins or to membrane components of cells. Others are such that, when activated, cleave one or more other complement proteins. Upon cleavage some of the complement proteins yield fragments that activate cells, increase vascular permeability or opsonize bacteria.

2. Coagulation system – Depending on the severity of the tissue injury, the coagulation system may or may not be activated. Some products of the coagulation system can contribute to the non-specific defenses because of their ability to increase vascular permeability and act as chemotactic agents for phagocytic cells. In addition, some of the products of the coagulation system are directly antimicrobial. For example, beta-lysin, a protein (Sabatino et al., 2012) produced by platelets during coagulation can lyse many Gram-positive bacteria by acting as a cationic detergent.

3. Lactoferrin and transferrin – Lactoferrin (formerly known as lactotransferrin) is a glycoprotein and a member of transferring family capable of binding and transferring iron (Fe^{3+} ions). It is therefore an iron chelator. By binding iron, an essential nutrient for bacteria, these proteins limit bacterial growth. It plays several biological roles and has antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant and immunomodulatory activities and it is part of the innate defense mainly at mucoses (Sanchez et al., 1992). It can be purified from

milk or produced recombinantly. Human colustrum has the concentration followed by human milk (Levin et al., 2006) then cow milk.

Laurell described a plasma iron transport protein that he called 'Transferrin' (Hillman, 2001). Transferrin like lactoferrin is an iron binding glycoprotein that constitutes 7.5 to 8% of bovine immunoglobulin similar to lactoferrin. It inhibits multiplication and growth of certain viral, bacterial and fungal organisms by iron inhibition.

4. Interferons – Interferons are potent cytokines that possess antiviral, immune modulating and antiprolifrerative actions (Baron et al., 1992). These proteins are synthesized by cells in response to various inducers and in turn cause biochemical changes leading to an antiviral state in cells of the same species (Hayden, 2001). Three major classes of human interferons with significant antiviral activity currently are recognized: alpha, beta and gamma (Hayden, 2001).

5. Lysozyme – Sir Alexander Fleming (1881-1955) discovered and named lysozyme in 1921 by Nobel Prize winner. Lysozyme also known as muramidase is a small antibiotic enzyme that kills bacteria by attacking their protective cell walls. They are glycoside hydrolases and are used in cell lysis, antimicrobial and food preservative applications. Lysozyme is part of the innate immune system (Revenis, and Kaliner, 1992). Since lysozyme is a natural form of protection from gram positive pathogens (Anderson and Nester, 2007); a deficiency due to infant formula feeding can lead to increase incidence of disease.

6. Interleukin-1 – This molecule is a highly potent initiator of inflammation and also induces fever and the production of acute phase proteins. The biologic response

modifier, anakinra blocks the action of interleukin-1 by acting as an inactive decoy, binding to its receptor and blocking the binding of interleukin-1 itself. It induces fever (Duff, 1985) and the production of acute phase proteins, some of which are antimicrobial, because they can opsonize bacteria.

Table 1: Physico-chemical barriers to infections

System / Organ	Active component	Effector Mechanism
Skin	Squamous cells; Sweat	Desquamation; flushing, organic acids
Gastro intestinal	Columnar cells	Peristalsis, low pH, bile acid, flushing,

tract		thiocyanate
Lung	Tracheal cilia	Mucociliary elevator, surfactant
Nasopharynx and eye	Mucus, saliva, tears	Flushing, lysozyme
Circulation and lymphoid organs	Phagocytic cells	Phagocytosis and intracellular killing
	NK cells and K-cell	Direct and antibody dependent cytolysis
	LAK	IL2-activated cytolysis
Serum	Lactoferrin and Transferrin	Iron binding
	Interferons	Antiviral proteins
	TNF-alpha	antiviral, phagocyte activation
	Lysozyme	Peptidoglycan hydrolysis
	Fibronectin	Opsonization and phagocytosis
	Complement	Opsonization, enhanced phagocytosis, inflammation

(Source: pathmicro.med.sc.edu/.innate.htm)

1.1.2.1.1.3 Cellular barriers

Part of the inflammatory response is the recruitment of eosinophils and macrophages to sites of infection. These cells are the main line of defense in the non-specific immune system.

1. Neutrophils–Polymorphonuclear cells (PMNs) are recruited to the site of infection where they phagocytose invading organisms and kill them intracellularly. In addition, PMNs contribute to collateral tissue damage that occurs during inflammation.

2. Eosinophils – Eosinophils 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. In normal individuals, eosinophils make up about 1-6% of white blood cells and are about 12-17 micrometers in size. Eosinophils persists in the circulation for 8-12 hours and can survive in tissus for an additional 8-12 days in the absence of stimulation (Young et al., 2006). Eosinophils are also involved in many other biological process, including post pubertal mammary gland development, oestrus cycling, allograft rejection and neoplasia (Rothenberg and Hogan, 2006).

3. Macrophages – Human macrophages are about 21µm in diameter (Khazan et al., 2005). They function in both non-specific defense as well as help initiate specific defence mechanisms (Krombach et al., 1997). Tissue macrophages and newly recruited monocytes which differentiate into macrophages also function in phagocytosis and intracellular killing of microorganisms. In addition, macrophages

are capable of extracellular killing of infected or altered self target cells. Furthermore, macrophages contribute to tissue repair and act as antigen-presenting cells, which are required for the induction of specific immune responses. These phagocytes engulf and kill microorganisms. They are able to travel outside of the circulatory system by moving across the cell membrane of blood vessels (Zen and Parkos, 2003). They contribute to tissue repair and can present antigens to elements of the adaptive immune system. Macrophages also secrete powerful chemicals that kill microorganisms and

can provoke inflammation.

4. Natural Killer (NK) and Lymphokine Activated Killer (LAK) cells – NK and LAK cells can non-specifically kill virus infected and tumor cells. These cells are not part of the inflammatory response but they are important in non-specific immunity to viral infections and tumor surveillance. They are able to differentiate between self and foreign by the presence or absence of major histocompatibility complex (MHC) -class I molecules (Vivier et al., 2011). Healthy cells express MHC class I molecules on their surface, but virus-infected and malignant cells greatly reduce their expression, so natural killer cells will eliminate the infected cells. These cells are an active subject of investigation because they are able to differentiate between self and nonself – a process that goes awry in autoimmune disease.

1.1.2.2 Adaptive or Acquired immunity

The adaptive immune system launches attacks specific to the invading pathogen and requires some time to tailor its custom-made response. The adaptive system

"remembers" antigens it has encountered and reacts more quickly and efficiently the next time that antigen is found, yet more slowly than the innate system. A further subdivision of adaptive immunity is characterized by the cellsinvolved wherein we have:

a) Humoral immunity which is the aspect of immunity that is mediated by secreted antibodies. The cells most ostensibly involved in antibody production are the B lymphocytes (Lauralee, 2004). Humoral immunity is active when the organism generates its own antibodies and passive when antibodies are transferred between individuals (Silverstein, 1989).

b) Cell mediated immunity in which T-lymphocytes alone provide protection. This is active when the organism's own T-cells are stimulated and passive when T-cells come from another organism (Silverstein, 1989).

Thus, lymphocytes are the major component of the adaptive immune response

1.1.2.2.1 Lymphocytes

A lymphocyte is a type of white blood cell in the vertebrate immune system (Goronzy and Weyand, 2007). It was not until the pioneering experiments of Gowans that lymphocytes were recognized as being essential to immunity (Gowans et al., 1962). Under the microscope, lymphocytes can be divided into large lymphocytes and small lymphocytes. Large granular lymphocytes include natural killer cells (NK cells). Small lymphocytes consist of T cells and B cells. Thus, the three major types of lymphocytes are T cells, B cells and natural killer (NK) cells.

1.1.2.2.1.1 Natural killer cells

NK cells are a part of the innate immune system and are differentiated from the common lymphoid progenitor generating B and T lymphocytes (Roitt et al., 2001). They do not express T-cell antigen receptors (TCR), pan T marker CD3 or surface immunoglobulins (Ig) B cell receptors but they usually express the surface markers CD16 and CD56 in humans, NK1.1 or NK1.2 in C57BL/6 mice (Kiessling et al., 1975). They were named natural killers because of the initial notion that they do not require activation in order to kill cells which lack "self" markers of major histocompatibility (MHC class 1) (Oldham, 1983). NK cells play a major role in defending the host from both tumors and virally infected cells. NK cells distinguish infected cells and tumors from normal and uninfected cells by recognizing changes of MHC 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.

1.1.2.2.1.2 T cells and B cells

T cells (thymus cells because they mature in the thymus (Alberts et al., 2002)) and B cells (bursa-derived cells) are the major cellular components of the adaptive immune response. All T cells originate from hemapoietic stem cells in the bone marrow. Hemapoietic progenitors derived from hemapoietic stem cells populate the thymus and expand by cell division to generate a large population of immature thymocytes (Schwarz and Bhandoola, 2006). The thymus contributes fewer cells as a person ages. As the thymus shrinks by about 3% a year throughout middle age ((Haynes et al., 2000)), there is a corresponding fall in the thymic production of

naive T cells, leaving peripheral T cell expansion to play a greater role in protecting older subjects.

T cells are involved in cell-mediated immunity whereas B cells are primarily responsible for humoral immunity. 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. There are several subsets of T cells, each with a distinct function, namely:

(a) Helper T cells- assist other white cells in immunologic processes, including maturation of B cells into plasma cell and memory B cells, and activation of cytotoxic T cells and macrophages. These cells are also known as CD4+T cells because they express the CD4 protein on their surface. Helper T cells become activated when they are presented with peptide antigens by MHC class II molecules, which are expressed on the surface of antigen presenting cells (APCs). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response. These cells can differentiate into one of several subtypes, including TH1, TH2, TH3, TH17, and TFH, which secrete different cytokines to facilitate a different type of immune response. Signaling from APCs directs T cells into particular subtypes.

(b) Cytotoxic T cells- destroy virally infected cells and tumor cells, and are also implicated in transplant rejection. These cells are also known as CD8+T cells since they express the CD8 glycoprotein at their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of nearly every cell of the body. Through IL-10, adenosine and other molecules secreted by regulatory T cells, the CD8+ cells can be inactivated to an anergic state, which prevent autoimmune diseases such as experimental autoimmune encephalomyelitis (Jiang and Chess, 2004).

(c) Memory T cells- are subset of antigen-specific T cells that persist after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with "memory" against past infections. Memory T cells comprise two subtypes: central memory T cells and effector memory T cells (Willinger et al., 2005). Memory cells may be either CD4+ or CD8+. Memory T cells typically express the cell surface protein CD45RO (Akbar et al., 1988).

(d) Regulatory T cells- formerly known as suppressor T cells are crucial for the maintenance of immunological tolerance. Their major role is to shut down T cell-mediated immunity toward the end of an immune reaction and to suppress auto-reactive T cells that escaped the process of negative selection in the thymus. Two major classes have been described: naturally occurring regulatory T cells which arise in the thymus. They have been linked to interactions between developing T cells with both myeloid and plasmacytoid dendritic cells that have been activated

with thymic stromal-derived lymphopoietin (TSLP). (Watanabe et al., 2005; Hanabuchi et al., 2010). Naturally occurring regulatory T cells can be distinguished from other T cells by the presence of an intracellular molecule called FoxP3. Mutations of the FoxP3 gene can prevent regulatory T cell development, causing the fatal autoimmune disease called IPEX (Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome). Adaptive regulatory T cells may originate during a normal immune response.

(e) Natural killer T cells (NKT cells) - They are not to be confused with natural killer cells of the innate immune cells. Unlike conventional T cells that recognize peptide antigens presented by major histocompatibility complex (MHC) molecules, NKT cells recognize glycolipid antigen presented by a molecule called CD1d (Mansour, 2008). Once activated, these cells can perform functions ascribed to both Th and Tc cells (i.e., cytokine production and release of cytolytic/ cell killing molecules). They are also able to recognize and eliminate some tumor cells infected with herpes viruses.

(f) Gamma delta (γδ) T cells- represent a small subset of T cells that possess a distinct T cell receptor (TCR) on their surface. A majority of T cells have a TCR composed of two glycoprotein chains called α- and β- TCR chains. However, in γδ T cells, the TCR is made up of one γ-chain and one δ-chain. This group of T cells is much less common (2% of total T cells) than the αβ T cells, but are found at their highest abundance in the gut mucosa, within a population of lymphocytes known as intraepithelial lymphocytes (IELs) (Holtmeier and Kabelitz, 2005). The antigenic

molecules that activate γδ T cells are still widely unknown. γδ T cells are believed to play a prominent role in recognition of lipid antigens. They are of an invariant nature and may be triggered by alarm signals, such as heat shock proteins (HSP). There also exists, a γδ T cells sub-population within the epidermal compartment of the skin of mice which was originally referred to as Thy 1+ Dendritic Epidermal cells (Thy 1 + DEC) (Bergstresser et al., 1985). However, γδ T cells are not MHC restricted and seem to be able to recognize whole proteins rather than requiring peptides to be presented by MHC molecules on antigen presenting cells.

1.1.2.2.2 B lymphocytes and Antibody Production

There are some basic cell-cell interactions that lead to antibody production. They are:

(a) Antigen Processing

When the macrophage engulfs bacteria, proteins (antigens) from the bacteria are broken down into short peptide chains and those peptides are displayed on the macrophage surface attached to major histocompatibility complex Class II (MHC II). Bacterial peptides are similarly processed and displayed on MHC II molecules on the surface of B lymphocytes.

(b) Helper T Cell Stimulating B cell.

When a T lymphocyte “sees” the same peptide on the B cell, the T cell stimulates the B cell to turn on antibody production.

(c) The stimulated B cell undergoes repeated cell divisions, enlargement and differentiation to form a clone of antibody secreting plasma cells (Kaiser, 2009).

Hence, through specific antigen recognition of the invader, clonal expansion and B cell differentiation, an effective number of plasma cells all secreting the same needed antibody which circulate in the body fluids via plasma and lymph is acquired. The antibody then binds to the bacteria making them easier to ingest by white cells. Antibody combined with a complement may also kill the bacteria directly. When the B cell fails in any step of the maturation process, it will die by apoptosis, here called “clonal deletion” (Parham, 2005).

1.1.2.2.3 Alternative adaptive immune system

Although the classical molecules of the adaptive immune system (e.g. antibodies and T cell receptors) exist only in jawed vertebrates, a distinct lymphocyte-derived molecule has been discovered in primitive jawless vertebrates, such as the lamprey and hagfish. These animals possess a large array of molecules called variable lymphocyte receptors (VLRs) that, like the antigen receptors of jawed vertebrates, are produced from only a small number (one or two) of genes. These molecules comprise the alternative adaptive immune system, and are believed to bind pathogenic antigens in a similar way to antibodies, and with the same degree of specificity (Alder et al., 2005).

1.1.2.2.4 Immunological Memory

Immunological memory is the capacity of the body's immune system to remember an encounter with an antigen due to the activation of B cells or T cells having specificity for the antigen and to react more swiftly to the antigen by means of these

activated cells in a later encounter. This leads to a perception that an individual is immune to a particular agent.

1.1.2.2.4.1 Passive memory

Passive memory is usually short-term, lasting between a few days and several months. Newborn infants have had no prior exposure to microbes and are particularly vulnerable to infection. Several layers of passive protection are provided by the mother. In the uterus, maternal IgG is transported directly across the placenta (Janeway et al, 2001), so that at birth, human babies have high levels of antibodies, with the same range of antigen specificities as their mother (Janeway et al, 2001). Breast milk contains antibodies that are transferred to the gut of the infant, protecting against bacterial infections, until the newborn can synthesize its own antibodies (Janeway et al, 2001). This is passive immunity because the fetus does not actually make any memory cells or antibodies, it only borrows them. Short-term passive immunity can also be transferred artificially from one individual to another via antibody-rich serum.

1.1.2.2.4.2 Active memory and immunization

Long-term active memory is acquired following infection by activation of B and T cells. Active immunity can also be generated artificially, through vaccination. The principle behind vaccination (also called immunization) is to introduce an antigen from a pathogen in order to stimulate the immune system and develop specific

immunity against that particular pathogen without causing disease associated with that organism (Alberts et al., 2002). This deliberate induction of an immune response is successful because it exploits the natural specificity of the immune system, as well as its inducibility. With infectious disease remaining one of the leading causes of death in the human population, vaccination represents the most effective manipulation of the immune system mankind has developed (Janeway et al., 2001). A vaccine stimulates a primary response against the antigen without causing symptoms of the disease. Most viral vaccines are based on live attenuated viruses, while many bacterial vaccines are based on acellular components of micro-organisms, including harmless toxin components (Alberts et al., 2002). Since many antigens derived from acellular vaccines do not strongly induce the adaptive response, most bacterial vaccines are provided with additional adjuvants that activate the antigen-presenting cells of the innate immune system and maximize immunogenicity (Singh et al., 1999).

1.1.2.3 Passive immunity

Passive immunity is the transfer of active immunity, in the form of readymade antibodies, from one individual to another. Passive immunity can occur naturally, when maternal antibodies are transferred to the fetus through the placenta (usually around the third month of gestation (Coico et al., 2003), and can also be induced artificially, when high levels of human (or horse) antibodies specific for a pathogen or toxin are transferred to non-immune individuals. Passive immunization is used when there is a high risk of infection and insufficient time for the body to develop its own immune response, or to reduce the symptoms of ongoing or

immunosuppressive diseases. Passive immunity provides immediate protection, but the body does not develop memory, therefore the patient is at risk of being infected by the same pathogen later.

1.1.2.3.1 Naturally acquired passive immunity

Maternal passive immunity is a type of naturally acquired passive immunity, and refers to antibody-mediated immunity conveyed to a fetus by its mother during pregnancy. Maternal antibodies (MatAb) are passed through the placenta to the fetus by an FcRn receptor on placental cells. This occurs around the third month of gestation (Hunt et al., 2004). IgG is the only antibody isotype that can pass through the placenta (Hunt et al., 2004) because IgM, IgD, IgE, IgA do not cross the placenta, they are almost undetectable at birth, although some IgA is provided in breast milk. (Silverstein, 1989). Passive immunity is also provided through the transfer of IgA antibodies found in breast milk that are transferred to the gut of the infant, protecting against bacterial infections, until the newborn can synthesize its own antibodies. These passively acquired antibodies can protect the newborn up to 18 months, but their response is usually short-lived and of low affinity (Gherardi, 2006). During adolescence, the human body undergoes several physical, physiological and immunological changes. These changes are started and mediated by different hormones. Depending on the sex, either testosterone or 17-β-oestradiol, act on male and female bodies not only on the primary and secondary sexual characteristics, but also have an effect on the development and regulation of the immune system.

1.1.2.3.2 Artificially acquired passive immunity

Artificially acquired passive immunity is a short-term immunization induced by the transfer of antibodies, which can be administered in several forms; as human or animal blood plasma, as pooled human immunoglobulin for intravenous (IVIG) or intramuscular (IMIG) use, and in the form of monoclonal antibodies (MAb). It provides immediate protection against an antigen, but does not provide long lasting protection (Goronzy and Weyand, 2007). There is a marked improvement in the body's response to polysaccharides from 12-24 months of age. This could be the reason for the specific time frames found in vaccination schedules (Al-Razi, 2003). Passive transfer is used prophylactically in the case of immunodeficiency diseases, such as hypogammaglobulinemia (Janeway et al., 2001). It is also used in the treatment of several types of acute infection, and to treat poisoning. Immunity derived from passive immunization lasts for only a short period of time, and there is also a potential risk for hypersensitivity reactions, and serum sickness, especially from gamma globulin of non-human origin. The artificial induction of passive immunity has been used for over a century to treat infectious disease, and prior to the advent of antibiotics, was often the only specific treatment for certain infections. For example immunoglobulin therapy was the only approach in the treatment of severe respiratory diseases until the 1930s, even after sulphonamide antibacterial agents were introduced (Janeway et al., 2001).

1.1.2.3.3 Passive transfer of cell-mediated immunity

Passive or "adoptive transfer" of cell-mediated immunity, is conferred by the transfer of "sensitized" or activated T-cells from one individual into another. It is

rarely used in humans because it requires histocompatible (matched) donors, which are often difficult to find. In unmatched donors, this type of transfer carries severe risks of graft versus host disease. It has, however, been used to treat certain diseases including some types of cancer and immunodeficiency. This type of transfer differs from a bone marrow transplant, in which (undifferentiated) hematopoietic stem cells are transferred.

1.1.3 Parts of the Immune System

In the 21st century, immunology has broadened its horizons with much research being performed in the more specialized niches. This includes the immunological function of cells, organs, (see Figure 2) and systems not normally associated with the immune system, as well as the function of the immune system outside classical models of immunity.

1.1.3.1 Organs of the Immune System

(a) Primary lymphoid organs

(i) Bone marrow: This is the site of hematopoiesis and B lymphocyte development. Bone marrow is a loosely-organized grouping of cells located in central soft tissue portion of bones (surrounded by the calcified matrix) throughout the body. Hematopoietic stem cells (HSC) present in the bone marrow are responsible for development of all blood cells after about the seventh month

of gestation in humans (Stevenson, 2011). B lymphocytes, granulocytes, monocytes and erythrocytes all develop to maturity in the bone marrow before they are released into the bloodstream for transport to other locations in the body.

(ii) Thymus: This is the site of T lymphocyte development. It is a flattened, grayish, bilobed organ that lies within a fat deposit together with the periaortic lymph node just anterior to the heart in the thoracic cavity. There are two types of tissue namely: reticular cells (stromal cells) and developing T lymphocytes (T cells) which are randomly situated in clusters throughout the thymus (Stevenson, 2011).

(b) Secondary lymphoid organs

(i) Lymph nodes: These are bean-shaped, encapsulated nodules located at junctions of lymphatics at strategic areas of the body. The lymph nodes filter particulate and soluble molecules out of lymph (interstitial tissue fluid picked up by the lymphatics for transport to the lymph ducts that empty into the subclavian veins), thus capturing immunogens for immune system stimulation. Three types of tissue (in addition to the ubiquitous reticular cells that form the tissue matrix along with trabecular connective tissue) are found in lymph nodes, namely:

- Cortical (cortex) tissue: This is located in the outer region of lymph nodes, just inside the subcapsular sinus (into which lymph drains from afferent lymphatics).B cells are the primary lymphoid cells here (but there are some T cells and follicular dendritic cells present as well). Lymphoid follicles characterize the cortical region of lymph nodes. Germinal centers develop within

lymphoid follicles as a result of antibody responses that occur here (Stevenson, 2011).

❖ Paracortical (paracortex) tissue: Located in the intermediate region of lymph nodes and partially surrounding lymphoid follicles (on the medullary side of the cortex). T cells are the primary lymphoid cells here (but there are some macrophages and dendritic cells present as well) but they migrate in and out of this tissue. They migrate into lymphoid follicles following antigenic stimulation by APCs to better "deliver" cytokines to B cells responding to immunogens present and they migrate from lymph into blood and from blood into lymph (via high endothelial venule cells in lymph nodes in both cases).

❖ Medullary (medulla) tissue: Located in the central region of lymph nodes as a loosely-organized aggregate of predominantly phagocytic cells. Macrophages and dendritic cells (both are APCs) are the primary lymphoid cells here (but there are variable numbers of plasma cells, especially during active immune responses).

(ii) Spleen: This is a lumpy, amorphous encapsulated lymphoid organ (much larger than a normal lymph node) located ventral to the stomach in the abdominal cavity. The spleen filters particulate and soluble molecules out of blood, thus capturing immunogens for immune system stimulation. Trabecular connective tissue forms the splenic matrix, which contains two major types of tissue namely red pulp and white pulp.

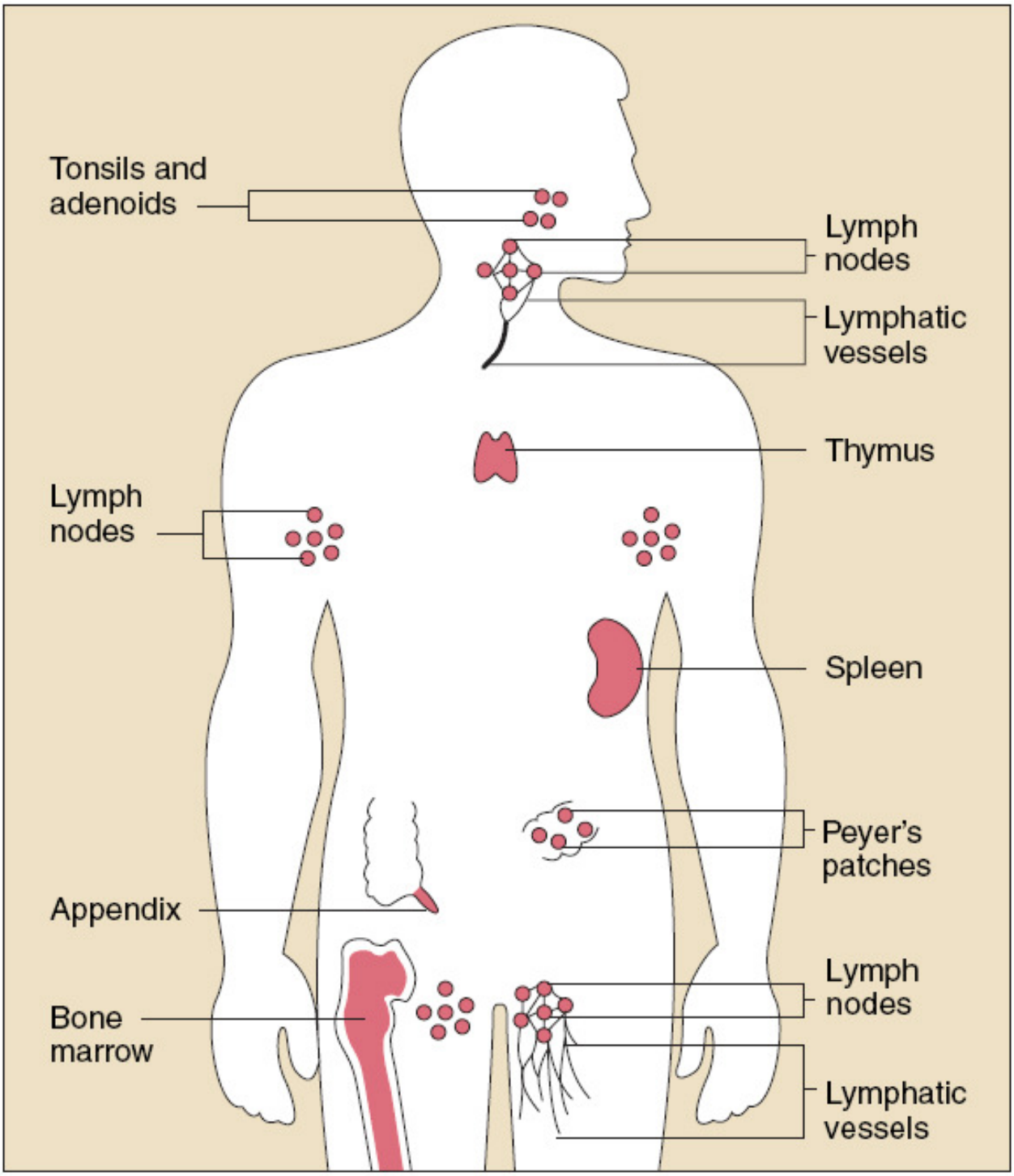
(iii) Mucosal-associated lymphoid tissue (MALT): This generally consists of rather loosely-organized lymphoid cells that are associated with mucosal tissues that line the digestive tract, including the tonsils, lamina propria and submucosal

lymphoid follicles of the small intestine, including Peyer's patches and appendix (Warwick and Williams, 1973).

(iv) Respiratory and urogenital tracts contain other, more loosely-organized lymphoid follicles.

(v) Cutaneous-associated lymphoid tissue of the epidermis contains intraepidermal lymphocytes and Langerhans (dendritic) cells which can process and present antigen to T cells. They migrate to local lymph nodes after they have phagocytosed exogenous antigen (presumably just so they can present their processed antigen to T cells).These organs are shown in Figure 2 below.

Figure 2: Organs of the immune system



(Source: **Error! Hyperlink reference not valid.** &Research Topics>Immune System)

1.1.3.2 Cells of the Immune System

Immunity is maintained in the body by a complex organization of cells of diverse morphology and distributed widely in different parts of the body. Cells of the immune system are associated with the lymphatic system of the body and are grouped into myeloid and lymphoid cells.

a) Myeloid Cells: The term myeloid suggests an origin in the bone marrow or spinal cord or a resemblance to the marrow or spinal cord. Myeloid cell is used to describe any blood cell that is not a lymphocyte. There are five groups of cells that make up the myeloid cells.

i) Granulocytes: Granulocytes comprise 41-81% of blood leukocytes. They have multilobed nucleus and are 20-30 μm in diameter with loosely packed strands of intensely basophilic chromatin surrounded by a moderate amount of lightly basophilic cytoplasm containing large numbers of granules. Granulocytes are further subdivided into neutrophils, eosinophils and basophils according to the staining properties of granules in their cytoplasm (Rang et al., 2007).

Neutrophils comprise 40-75% of blood leukocytes. Neutrophils are the shock troops of inflammation and are the first of the blood leucocytes to enter an inflamed area (Rang et al., 2007). They contain small, lightly staining granules and are phagocytic effectors of antibody-mediated immunity and hypersensitivity.

Eosinophils comprise 1-5% of blood leukocytes. They contain orange-to-red staining granules and they help to regulate inflammatory responses. They are active in antibody-mediated cytolysis of immature forms of intestinal parasites.

Basophils comprise only 0.5% of blood leukocytes (Rang et al., 2007). They contain large, blue-black staining histamine-rich cytoplasmic granules and Fc receptor that bind IgE molecules. They help generate inflammatory responses and are mediators of immediate (type I) hypersensitivity.

ii) Mast Cells are cells found in connective tissue that contains numerous basophilic granules and releases substances such as heparin and histamine in response to injury or inflammation of body tissues. They are tissue cells with histamine-rich cytoplasmic granules and FcR that bind IgE molecules (approximately ten times more than basophils). They help generate inflammatory responses (Krishnaswamy et al, 2006) and are tissue mediators of immediate (type I) hypersensitivity. They are also associated with allergy and anaphylaxis (Stvrtinova et al., 1995).

iii) Monocytes comprise 3-7% of blood leukocytes. They are 20-50 μm in diameter with large and indented nucleus. They contain loosely packed strands of intensely basophilic chromatin surrounded by a large amount of lightly basophilic cytoplasm (which contains numerous granules and occasional vacuoles. They engulf and digest foreign matter (Janeway, 2005), thus they are phagocytic effectors of cell-mediated immunity and hypersensitivity.

iv) Macrophages are derived from monocytes after they migrate into tissues (Rang et al., 2007) (e.g. histiocytes in connective tissue, alveolar macrophages in lung, microglial cells in central nervous system, mesangial cells in kidney, Kupffer cells in liver, osteoclasts in bone, etc.). The nucleus is large and indented, and contains loosely packed strands of intensely basophilic chromatin surrounded by a large amount of lightly basophilic cytoplasm. The cytoplasm contains numerous granules and vacuoles, especially when activated by T lymphocyte cytokines, such as interferon-gamma. Macrophages engulf and digest foreign matter. They are active in antigen processing and presentation and are also phagocytic effectors of cell-mediated immunity and hypersensitivity. They are also scavengers.

v) Dendritic Cells are covered with long membrane extensions that make them look like dendrites in nervous tissues, hence the name. During the adaptive immune response, antigen is presented to T cells by large dendritic cells (Rang et al., 2007). There are the following types:

- ❖ Circulating dendritic cells constitute 0.1% of blood leukocytes and are also found in lymph and develop into mature tissue dendritic cells.
- ❖ Interdigitating dendritic cells are found in T cell rich regions of secondary lymphoid tissues. They process and present antigen to T cells.
- ❖ Interstitial dendritic cells are found in most organs example lungs, liver, heart, kidney, digestive tract, etc. They process and present antigen to T cells (Guermonprez et al., 2002).

- ❖ Langerhans cells are found in epidermis of the skin. They process and present antigen to T cells

b) Lymphoid Cells

These cells comprise 20-45% of blood leukocytes and are responsible for immune responses. They are 10-30 µm in diameter with nearly round nucleus and contain coarse lumps of intensely basophilic chromatin. Their cytoplasm is lightly basophilic and variable in amount i.e. less cytoplasm in "resting" lymphocytes and more in "activity" lymphocytes. They include:

- ❖ B Lymphocytes- develop in bone marrow and differentiate into plasma cells, which synthesize and secrete antibody molecules. They are also capable of antigen processing and presentation.
- ❖ T Lymphocytes- develop in thymus. The T helper cells (Th) can synthesize and secrete cytokines and function to regulate immune responses (both antibody and cell-mediated) when appropriately stimulated during immune responses. The T cytotoxic cells (Tc) are mature precursor cells that differentiate into cytotoxic T lymphocytes (CTL) which mediate cellular immunity in virus-infected cells and tumor cells.

- ❖ Natural Killer (NK) Cells- develop in several lymphoid tissues, some in thymus, and others in bone marrow. They are not antigen-specific, but can recognize "self" cells. They are active in early phases of cell-mediated immune responses, synthesizing and secreting cytokines that promote

these responses. They kill targets (e.g. virus-infected or tumor cells) that lack ligands for inhibitory receptors on the NK cells themselves (Rang et al., 2007).

1.1.4 Pattern Recognition Receptors

Pattern recognition receptors (PRRs) are a primitive part of the immune system and are involved in the important initiating event in the innate immune response (Medzhitov and Janeway, 2000; Brown, 2001). They are also called primitive pattern recognition receptors because they are an early part of the immune system to evolve, before adaptive immunity. They are proteins expressed by cells of the innate immune system to identify pathogen-associated molecular patterns (PAMPs). PAMPs are associated with microbial pathogens or cellular stress, as well as damage-associated molecular patterns (DAMPs), which are associated with cell components released during cell damage. In addition to their role in innate immunity, toll-like receptors and other pattern-recognition receptors activate antigen-presenting cells and bridge innate and adaptive immunity by coordinating responses of T cells and B cells (Iwasaki and Medzhitov,2010; Netea et al., 2004). PRRs are classified according to their ligand specificity, function, localization and/or evolutionary relationships. On the basis of function, PRRs may be divided into signaling or endocytic PRRs.

1.1.5 Bystander T cell Apoptosis

Bystander T cell refers to T cells that are present but not involved in an immune response. Bystander activation of T cells refers to activation of T cells specific for an antigen X during an immune response against antigen Y (Suwannasaen et al.,

2010). Such an activation of T cells is independent of T-Cell Antigen Receptor (TCR) signaling and occurs through cytokines by novel activating receptors. Bystander activation of T cells has been reported in models of viral infections such as herpes simplex virus, lymphocytic choriomeningitis virus (LCMV) and human immunodeficiency virus (HIV) leading to proliferation of memory T cells and subsequent production of cytokines.

Studies have also found Bystander CD8+ T cell activation in response to intracellular bacteria. Using HIV as an example, Human immunodeficiency virus type 1 (HIV-1) infection causes functional impairment and progressive loss of $CD4^+$ T cells, leading to Acquired Immune Deficiency Syndrome (AIDS) (McCune, 2001). Apoptosis of uninfected Bystander CD+ T cells contributes to T cell depletion during human immunodeficiency virus type 1 pathogenesis. The viral host mechanism that lead to Bystander apoptosis are not well understood. Direct lysis of infected $CD4^+$ T cells and apoptosis of bystander cells occur during HIV-1 replication in vitro (Cao et al., 1996; Glushakova et al., 1998; Grivel and Margolis, 1999; Herbein et al., 1998; Jekle et al., 2003). Properties of the viral envelope glycoprotein that influence the ability of HIV- 1 to induce bystander apoptosis were investigated by Holm et al. (2004) using molecularly cloned viruses that differ only in specific amino acids. The results demonstrated that HIV-1 virions induce apoptosis through a C-X- chemokine receptor 4 (CXCR4) or C-Chemokine receptor 5 (CCR5) dependent pathway that does not require Env/CD4 signaling or membrane fusion and suggest that HIV-1 variants which increase envelope/receptor

affinity or co-receptor binding site exposure may promote T cell depletion *in vivo* by accelerating bystander cell death.

1.2 Disorders of human immunity

The immune system is a remarkably effective structure that incorporates specificity, inducibility and adaptation. The body's capability to react to antigen depends on a person's age, antigen type, maternal factors and the area where the antigen is presented (Silverstein, 1989). However, failures of host defense do occur and fall into three broad categories: immunodeficiencies, autoimmunity, and hypersensitivities.

1.2.1 Immunodeficiencies

Neonates are said to be in a state of physiological immunodeficiency, because both their innate and adaptive immunological responses are greatly suppressed or underdeveloped (Silverstein, 1989). Once born, a child's immune system responds favorably to protein antigens while not as well to glycoproteins and polysaccharides since B cells develop early in gestation but are not fully active (Gherardi, 2006). In fact, many of the infections acquired by neonates are caused by low virulence organisms like *Staphylococcus* and *Pseudomonas* (Silverstein, 1989). Immunodeficiencies occur when one or more of the components of the immune system are inactive. The ability of the immune system to respond to pathogens is diminished in both the young and the elderly, with immune responses beginning to decline at around 50 years of age due to immunosenescence (Aw et al., 2007; Chandra, 1997). In developed countries, obesity, alcoholism, and drug use are common causes of poor immune function (Chandra, 1997). However, malnutrition

is the most common cause of immunodeficiency in developing countries (Chandra, 1997; Niedzwiecki et al., 2005). Diets lacking sufficient protein are associated with impaired cell-mediated immunity, complement activity, phagocyte function, IgA antibody concentrations, and cytokine production. Additionally, the loss of the thymus at an early age through genetic mutation or surgical removal results in severe immunodeficiency and a high susceptibility to infection (Miller, 2002). Immunodeficiencies can also be inherited or 'acquired' (Alberts et al., 2002). Chronic granulomatous disease, where phagocytes have a reduced ability to destroy pathogens, is an example of an inherited, or congenital, immunodeficiency. HIV and some types of cancer cause acquired immunodeficiency (Joos and Tamm, 2005; Copeland and Heeney, 1996).

1.2.2 Autoimmunity

Overactive immune responses comprise the other end of immune dysfunction, particularly the autoimmune disorders. Here, the immune system fails to properly distinguish between self and non-self, and attacks part of the body. Under normal circumstances, many T cells and antibodies react with “self” peptides (Miller, 1993). One of the functions of specialized cells (located in the thymus and bone marrow) is to present young lymphocytes with self antigens produced throughout the body and to eliminate those cells that recognize self-antigens, preventing autoimmunity (Sproul et al., 2000). There is an increased risk in developing autoimmunity for pubescent and post pubescent females and males.

1.2.3 Hypersensitivity

Hypersensitivity is an immune response that damages the body's own tissues. They are divided into four classes (Type I – IV) based on the mechanisms involved and the time course of the hypersensitive reaction but in 1963, it was expounded to 5 groups (Gell and

Coombs, 1963).

Type I hypersensitivity is an immediate or anaphylactic reaction, often associated with allergy. Symptoms can range from mild discomfort to death. Type I hypersensitivity is mediated by IgE, which triggers degranulation of mast cells and basophils when cross-linked by antigen (Ghaffar, 2006). This can be seen in hay fever, initial phase of asthma and

urticaria (Rang et al., 2007).

Type II hypersensitivity occurs when antibodies bind to antigens on the patient's own cells, marking them for destruction. This is also called antibody-dependent (or cytotoxic) hypersensitivity, and is mediated by IgG and IgM antibodies (Ghaffar, 2006).

http://en.wikipedia.org/wiki/Immune_system_-_cite_note-USCH-73Immune complexes (aggregations of antigens, complement proteins, and IgG and IgM antibodies) deposited in various tissues trigger *Type III hypersensitivity* reactions (Ghaffar, 2006).

Type III hypersensitivity occurs when antibodies react with soluble antigens. The antigen-antibody complexes can activate complement or attach to mast cells and stimulate the release of mediators. This is seen in certain types of autoimmune kidney and arterial diseases and lupus erythematosus (Rang et al., 2007).

Type IV hypersensitivity (also known as cell-mediated or delayed type hypersensitivity) usually takes between two and three days to develop (Black, 1999). Type IV reactions are involved in many autoimmune and infectious diseases, but may also involve contact dermatitis (caused by e.g. poison ivy) and multiple sclerosis (Mitchelle et al., 2007). These reactions are mediated by T cells, monocytes, and macrophages (Ghaffar, 2006). The prototype of type IV hypersensitivity is the tuberculin reaction, a local inflammatory response seen when proteins derived from cultures of the tubercule bacillus are injected into the skin of a person who has been sensitised by a previous infection or immunization (Rang et al., 2007).

Type V hypersensitivity is an additional type that is sometimes used as a distinction from type II hypersensitivity (Regan, 2003). Instead of binding to cell surface components, the antibodies recognize and bind to the cell surface receptors, which either prevent the intended ligand binding with the receptor or mimic the effects of the ligand, thus impairing cell signaling.

1.3 Immunomodulation

This is a change in the body's immune system, caused by agents (immunomodulators) that activate or suppress its function. It is the adjustment of the immune response to a desired level, and involves immunostimulation, immunodepression, or induction of immunologic tolerance. Many proteins, amino acids, and natural compounds have shown a significant ability to regulate immune responses for example interferon-ɤ, steroids etc (Priyanka et al., 2012). Immunomodulators are classified into:

1.3.1 Immunosuppressants

Inhibit immune response in organ transplantation and autoimmune diseases. These drugs have major role in organ transplantation and autoimmune diseases (Rang et al., 2007). They include:

1. Calcineurin inhibitors (Specific T-cell inhibitors) - They include cyclosporine, tacrolimus.

Mechanism of action: Calcineurin inhibitors preferentially inhibit antigen-triggered signal transduction in T lymphocytes, blunting expression of many lymphokines as well as expression of anti-apoptotic proteins. Cyclosporine also forms a complex with cyclophilin, a cytoplasmic receptor protein present in target cells. This complex binds to calcineurin, inhibiting Ca^{2+} stimulated dephosphorylation of the cytosolic component of NFAT (nuclear factor of activated T cells) (Schreiber and Crabtree, 1992).

Clinical uses: Clinical indications for cyclosporine are organ transplantation for example kidney, liver and heart transplantation, rheumatoid arthritis and psoriasis (Faulds et al., 1993). It is generally considered as the agent that ushered in the modern era of organ transplantation, increasing the rates of early engraftment, extending graft survival for kidneys, and making cardiac and liver transplantation possible. Cyclosporine is usually used in combination with other agents, especially glucocorticoids and either azathioprine or mycophenolate mofetil and most recently sirolimus. Tacrolimus is indicated for the prophylaxis of solid-organ allograft rejection in a manner similar to cyclosporine and as rescue therapy in patients with rejection episodes despite therapeutic levels of cyclosporine (Mayer et al., 1997).

Side effects: The principal adverse reactions to cyclosporine therapy are renal dysfunction, tremor, hirsutism, hypertension, hyperlipidemia and gum hyperplasia (Burke et al., 1994). Tacrolimus causes neurotoxicty (tremor, headache, motor disturbances and seizures) and nephrotoxicity like cyclosporine in addition to gastrointestinal complaints, hypertension, hyperkalemia, hyperglycaemia and diabetes.

2. Antiproliferative and Antimetabolite drugs (Cytotoxic drugs)- They include sirolimus, azathioprine, cyclophosphamide, methotrexate, chlorambucil and mycophenolate mofetil (MMF).

Mechanism of action: Sirolimus inhibits T-lymphocyte activation and proliferation downstream of the IL-2 and other T-cell growth factor receptors (Kuo et al., 1992). Azathioprine, following exposure to nucleophiles is cleaved to 6-mercaptopurine, which, in turn, is converted to additional metabolites that inhibit new purine synthesis. (Bertino, 1973). A fraudulent nucleotide, 6-thio-IMP, is converted to 6-thio-GMP and finally to 6-thio-GTP, which is incorporated into DNA and gene translation is inhibited (Chan et al., 1987). Mycophenolate mofetil is rapidly hydrolysed to mycophenolic acid (the active form), which is a selective, uncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDG) (Natsumeda and Carr, 1993), an important enzyme in the new pathway of guanine nucleotide synthesis thus inhibiting B and T lymphocyte cell proliferation since they are highly dependent on this pathway for cell proliferation.

Clinical uses: Sirolimus is indicated for prophylaxis of organ transplant rejection in combination therapy with a calcineurin inhibitor and glucocorticoids (Kahan et al.,

1999a). In patients experiencing or at high risk for calcineurin inhibitor-associated nephrotoxicity, sirolimus has been used with glucocorticoids and mycophenolate mofetil to avoid permanent renal damage. Azathioprine was first introduced as an immunosuppressive agent in 1961, helping to make allogeneic kidney transplantation possible. It is indicated as an adjunct for prevention of organ transplant rejection and in severe rheumatoid arthritis (Hong

and Kahan, 2000; Gaffney and Scott, 1998).

Mycophenolate mofetil is indicated for prophylaxis of transplant rejection and is typically used in combination with glucocorticoids and a calcineurin inhibitor, but not with azathioprine (Kimball et al., 1995; Ahsan et al., 1999; Kreis et al., 2000).

Cyclophosphamide is an essential component of many effective drug combinations for non-Hodgkin's lymphomas. Complete remissions and presumed cures have been reported when cyclophosphamide was given as a single agent for Burkitt's lymphoma. It is frequently used in combination with methotrexate (or doxorubicin) and fluorouracil as adjuvant therapy after surgery for carcinoma of the breast (Chabner et al., 2001).

Chlorambucil is a standard agent for patients with chronic lymphocytic leukemia and primary (waldenstroms) macroglobulinemia (Chabner et al., 2001).

Methotrexate has been used in the treatment of severe disabling psoriasis. It is also used intermittently at low dosage to reduce remission in refractory rheumatoid arthritis (Hoffmeister, 1983). Complete awareness of the pharmacology and toxic potential of methotrexate is a prerequisite for its use in these non-neoplastic disorders (Weistein, 1977).

Side effects: Sirolimus' use in renal transplant patients causes a dose dependent increase in serum cholesterol and triglycerides that may require treatment. Others are lymphocoele, anaemia, hypokalemia or hyperkalemia, fever, gastrointestinal effects, increased risk of neoplasm especially lymphomas and infections. Azathioprine causes bone marrow suppression with leucopenia, thrombocytopenia and/or anaemia (Alan et al., 2001). Increase susceptibility to infections, hepatotoxicity, alopecia, gastrointestinal toxicity, pancreatitis and increase risk of neoplasia. Mycophenolate mofetil causes gastrointestinal and hematologic side effects including leucopenia, diarrhea, vomiting (Alan et al., 2001). There is incidence of some infections especially sepsis associated with cytomegalovirus.

3. Glucocorticoids-Prednisolone and others:

Mechanism of action: Steroids lyse and possibly induce the redistribution of lymphocytes, causing a rapid decrease in peripheral blood lymphocyte counts. Also, glucocorticoid-receptor complexes increase protein expression thereby curtailing activation of NFkB which results in increase in apoptosis of activated cells (Auphan et al., 1995), inhibition of cytotoxic T lymphocytes activation, and poor chemotaxis by neutrophils and monocytes. Thus they have broad anti-inflammatory effects on cellular immunity and relatively little effect on humoral immunity.

Clinical uses: These are commonly used in combination with other immunosuppressive agents both to prevent and treat transplant rejection. There are numerous indications for glucocorticoids (Zoorob and Cender, 1998). They are

efficacious for treatment of graft-versus-host disease in bone-marrow transplantation. Among autoimmune disorders, glucocorticoids are used routinely to treat rheumatoid arthritis, systemic lupus erythematosus, systemic dermatomyositis, psoriasis and other skin conditions, asthma and other allergic disorders, inflammatory bowel disease, inflammatory ophthalmic disease, autoimmune hematological disorders, and acute exacerbations of multiple sclerosis. In addition, glucocorticoids limit allergic reactions that occur with other immunosuppressive agents and are used in transplant recipients, to block first-dose cytokine storm caused by treatment with muromonad-CD3 (Alan et al., 2001).

Side effects: The extensive use of steroids has resulted in disabling and life-threatening adverse effects in many patients. These effects include growth retardation, avascular necrosis of bone, osteoporosis (Lane and Lukert, 1998), increased risk of infection, poor wound healing, cataracts, hyperglycemia hypertension and behavioural changes (Hasket, 1985).

4. Antibodies-Muromonab CD3, Antithymocyte globulin (ATG), Rho (D) immune globulin, Efalizumab (Rang et al., 2007).

Mechanism of action: Antithymocyte globulin contains cytotoxic antibodies that bind to CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, and HLA class 1 and 11 molecules on the surface of human T lymphocytes (Bourdage and Hamlin, 1995). The antibodies deplete circulating lymphocytes by direct cytotoxicity and block lymphocyte function.

Monoclonal antibodies induce rapid internalization of T-cell receptor, thereby preventing subsequent recognition of antigen. Hemagglutination generation

antibodies bind with high affinity to the alpha subunit of the IL-2 receptor present on the surface of activated, but not resting, T lymphocytes and block IL-2-mediated T-cell activation events (Krensky et al, 2001). Infliximab binds with high affinity to TNF-α and prevents cytokine from binding to its receptors (Krensky et al, 2001).

Clinical uses: Antithymocyte globulin is indicated for induction of immunosuppression and treatment of acute renal transplant rejection in combination with other immunosuppressive agents (Mariat et al., 1998).

Monoclonal antibodies (e.g. Muromonab-CD3) are indicated for treatment of acute organ transplant rejection (Ortho Multicenter Transplant Group, 1985; Woodle et al., 1999; Rostaing et al., 1999). Anti-IL-2-Receptor (Anti-CD25) antibodies are recommended for acute organ rejection in adult patients as part of combination therapy (with glucocorticoids, a calcineurin inhibitor, with or without azathioprine or mycophenolate mofetil) (Kovarik et al., 1999; Hong and Kahan, 1999; Kahan et al., 1999b; Hirose et al., 2000).

Several agents are used simultaneously (Krensky et al., 1990; Hong and Kahan, 2000) to achieve immunosupression.

Side effects: Antithymocyte globulin causes fever and chills with the potential for hypotension, (Alan et al., 2001) glomerulonephritis, hematologic complications include leucopenia, thrombocytopenia. Monoclonal antibodies may cause anaphylactic reactions, lympho-proliferative disorders and opportunistic infections (Alan et al., 2001).

1.3.2 Immunostimulants

They increase the immune response; they are useful in infections, immunodeficiency (for example, AIDS) and cancers. They stimulate the immune

system to fight against immunodeficiencies (like AIDS), infections and cancers. They include:

1. Levamisole- is an antihelmintic drug that also restores functions of B lymphocytes, T lymphocytes, monocytes and macrophages.

Clinical uses: Its only clinical indication is as an adjuvant treatment with fluorouracil after surgical resection in patients with Duke's stage C colon cancer (Moertel et al., 1990;

Figueredo et al., 1997).

Mechanism of action: Levamisole acts by elevating cGMP levels in lymphocytes and enhance their proliferative response to nitrogen or foreign cells. Its imidazole ring seems to be one of the active moieties responsible for the functional increase of peripheral T-cell and macrophages (Alan et al., 2001).

Side effects include leucopenia, agranulocytosis, transient granulocytopenia, skin rash, dermatitis, alopecia, pruritus, urticaria and flu-like symptoms including fatigue, fever, rigors, myalgia, and malaise and rarely, encephalopathy (Rang et al., 2007).

2. Thalidomide-Thalidomide is best known for the severe, life-threatening birth defects it caused when administered to pregnant women (Smithells and Newman, 1992; Lary et al., 1999).

Mechanism of action: It has been reported to decrease circulating TNF-α in patients with erythema nodosum leprosum (ENL) but increases it in patients who are HIV-seropositive (Jacobson et al., 1997). Alternatively, it has been suggested that the drug affects angiogenesis (Miller and Stromland, 1991).

Clinical uses: Different effects of this old drug have been utilized in conditions such as:

a) Erythema nodosum leprosum (ENL) ((Sampaio et al., 1993): Anti-inflammatory effect

b) Multiple myeloma: Anti-angiogenesis

c) Rheumatoid arthritis: Anti TNF effect.

Side effects include birth defects e.g. phocomelia. Others are polyneuropathy, fatigue, skin rash and venous thromboembolism.

3. Bacillus Calmette Guerin (BCG) - Live BCG is an attenuated, live culture of the bacillus of Calmette and Guerin strains of *Mycobacterium bovis* (Alan et al., 2001). It is a biologic response modifier.

Mechanism of action: The exact mechanism of action is yet to be elucidated; however it appears that it is mediated by the local immune response mainly through T-helper cell response (Kapoor et al., 2008).

Clinical uses: It is active against tumours; hence it is indicated for treatment and prophylaxis of carcinoma *in situ* of the urinary bladder and for prophylaxis of primary and recurrent stage of papillary tumors following transurethral resection (Morales et al., 1981; Paterson and Patel, 1998; Patard et al., 1998).

Side effects: include painful or difficult urination, urinary urgency and frequency, blood in urine, and flu-like symptoms such as fever, chills, malaise, fatigue, generalized aches and pains.

4. Recombinant cytokines

a) Interferon - Although interferon (alpha, beta, and gamma) initially were identified by their antiviral activity, these agents have important immunomodulatory activities as well (Johnson et al, 1994; Tilg and Kaser, 1999; Ransohoff, 1998).

Mechanism of action: Interferons bind to specific cell surface receptors that initiate a series of intracellular events: induction of certain enzymes, inhibition of cell proliferation, and enhancement of immune activities, including increased phagocytosis by macrophages and augmentation of specific cytotoxicity by T lymphocytes (Tompkins, 1999). Interleukin-2 enhances lymphocyte proliferation and growth of IL-2 dependent cell-lines, enhances lymphocyte mediated cytotoxicity and induces interferon-gamma activity. (Winkelhake et al., 1990; Whittington and Faulds, 1993).

Clinical uses: Interferon alfa-2b: This is indicated in the treatment of a variety of tumors, including hairy cell leukemia, malignant melanoma, follicular lymphoma, and AIDS-related Kaposi's sarcoma (Punt, 1998; Bukowski, 1999; Sinkovics and Horvath, 2000). It is also indicated for infectious diseases, chronic hepatitis B, and condylomata acuminata. In addition, it is supplied in combination with ribavirin for the treatment of chronic hepatitis C in patients with compensated liver function not treated previously with interferon alfa-2b or who had a relapse following interferon alfa-2b therapy (Lo Iacono et al., 2000).

Interferon gamma-1b: This is indicated in reducing the frequency and severity of serious infections associated with chronic granulomatous disease (Alan et al., 2001).

Interferon beta-1a: This is FDA-approved for the treatment of relapsing and

relapsing-remitting multiple sclerosis to reduce the frequency of clinical exacerbations (Alan et al., 2001).

Interleukin-2: This is indicated for the treatment of adults with metastatic renal cell carcinoma and melanoma (Alan et al., 2001).

Side effects: Recombinant cytokines are capable of causing cancer.

1.3.3 Tolerogens

Tolerogens induce tolerance and make the tissue non-responsive to antigen; tolerance is the state of non-responsiveness to antigen. The approaches used to induce tolerance include:

1. Costimulatory blockade- Induction of specific responses by T lymphocytes requires 2 signals: an antigen specific signal via the T-cell receptor and a costimulatory signal provided by molecules such as CD28 on the T-cell interacting with CD80 and CD86 on the antigen-presenting cell (APCs) (Khoury et al., 1999). Preclinical studies have shown that inhibition of the co-stimulatory signal can induce tolerance (Larsen et al., 1996; Kirk et al., 1997). Recombinant fusion protein molecules, anti CD50 MAB and anti CD56 MAB act by this mechanism (Krensky et al., 2001). The long and torturous road in the clinical development of costimulatory blockade came to fruition in 2005, with the approval of CTLA4Ig (abatacept) for rheumatoid arthritis and for the publication of the promising results of the phase II trial in kidney transplantation of balatacept previously referred to as LEA29Y (Vincenti, 2006).

2. Donor cell chimerism-Another approach is induction of chimerism (coexistence of two genetic lineages in a single individual) by any variety of protocols that first dampens or eliminates immune function in the recipient (this is done with ionizing

radiation, drugs such as cyclophosphamide, and/or antibody treatment), then a hemagglutination source of immune function by adoptive transfer (transfusion) of bone marrow or hematopoietic stem cells is instituted (Starzl et al., 1997; Fuchimoto et al., 1999; Spitzer et al., 1999; Hale et al., 2000). Upon reconstitution of immune function, the recipient no longer recognizes hemagglutination antigens provided during a critical period as "nonself." Such tolerance is long-lived and is less likely to be complicated by the use of calcineurin inhibitors (Alan et al., 2001). Despite improved immunosuppressive drug therapy, patients are faced with substantial side effects and the risk of chronic rejection with subsequent graft loss. The transplantation of donor bone marrow for the induction of mixed chimerism has been recognized to induce donor-specific tolerance a long time ago, but safety concerns regarding toxicities of current bone marrow transplantation protocols impede widespread application (Pilat et al., 2012).

3. Soluble HLA-Human leukocyte antigen induces tolerance through blood. In the precyclosporine era, blood transfusion was shown to be associated with improved outcomes in renal transplant patients (Opelz and Terasaki, 1978). These findings gave rise to donor-specific transfusion protocols that gave improved outcomes (Opelz et al., 1997). After the introduction of cyclosporine, however, these effects of blood transfusion disappeared, presumably due to the efficacy of this drug in blocking T-cell activation. Nevertheless, the existence of a tolerance-promoting effect of transfusion is irrefutable. It is possible that this effect is due to HLA molecules on the surface of cells or in soluble forms. Recently, soluble HLA and peptides corresponding to linear sequences of HLA molecules have been shown to

induce immunologic tolerance in animal models via a variety of mechanisms (Murphy and Krensky, 1999). Soluble HLA-G modulates the immune tolerance of the mother and can be used as a prognostic factor for the clinical pregnancy rate. It also promotes successful implantation and pregnancy by modulating trophoblast invasion through receptor binding and activation of extracellular signal-regulated protein kinase signaling pathway (Guo et al., 2013).

4. Antigens: Specific antigens provided in a variety of forms but generally as peptides, induce immunologic tolerance in preclinical models of diabetes, arthritis and multiple sclerosis. Clinical trials of such approaches are under way. It is now well established that antigen / MHC complex binding to the T-cell receptor / CD3 complex coupled with soluble and membrane-bound costimulatory signals initiate a cascade of signaling events that lead to productive immunity. This approach is used to reverse autoimmune diabetes (Takiishi et al., 2012).

Mechanism of action: Costimulatory blockade – Preclinical studies have shown that inhibition of co-stimulatory signal induces tolerance (Larsen et al., 1996; Kirk et al; 1997). Antigen*s* target primary T-cell receptor-mediated signal either by blocking cell-surface receptor interaction/ inhibiting early signal transduction events. Clinical uses: For costimulatory blockade, two anti-CD154 monoclonal antibodies are under clinical evaluation in organ transplantation and autoimmunity. In general, tolerogens are used to treat the vast array of immune disorders, from autoimmunity to transplant rejection (Krensky et al., 2001).

Side effects: Long term protected recipients harbor T-cell capable of causing diabetes and immune tolerance.

1.3.4 Medicinal plants with Immunomodulatory effect

Search for better agents exerting immunomodulatory activity is becoming the field of major interest all over the world (Patwardhan et al., 1990). Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulatory agents, but there are major limitations to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system (Diasio and LoBuglio, 1996). Thus, the modulation of immune response in order to alleviate disease is of primary interest. Some medicinal plants, including *Murdannia loriformis* (Intayot et al., 2002), *Cymbopogon citrates* (Vinitketkumnuen et al., 1994), *Momordica charantia* (Vinitketkumnuen et al.,1996*)*, *Centella asiatica (*Farnsworth and Bunyapraphatsara, 1992*;* Babu et al., 1995), *Allium sativum* (Rueter*,* 1995; Yang et al., 1994), *Carthamus tinctorius* (Vinitketkumnuen et al., 1994), *Eclipta alba*, *Cyperus rotundus*, *Nelumbo nucifera* (Dee-Buo), and its embryos (Ke-Sorn-Buo), were found to have immunomodulatory effect on the mitogen stimulated proliferation of human peripheral blood mononuclear cells (Punturee et al., 2005). *Silybum marianum, Matricaria chamomilla, Calendula officinalis, Cichorium intybus and Dracocephalum kotschyi* which grow in Iran, were found to elicit immunomodulatory effect (Amirghofran et al., 2000). Below is the list of plants with immunomodulatory effect including the part(s) used (Table 2).

Table-2: List of some plants that have immunomodulatory activity

Plant	**Family**	**Part used**
Boerhaavia diffussa	Nyctaginaceae	Root
Curcuma longa	Zingiberaceae	Rhizome
Rhododendron spiciferum	Ericaceae	Leaf
Caesalpinia bonducella	Caesalpiniaceae	Whole plant
Tinospora cordifolia	Menispermaceae	Whole plant
Capparis zeylanica	Capparidaceae	Whole plant
Luffa cylindrical	Cucurbitaceae	Seed and fruit (bulb)
Withania somnifera	Solanaceae	Whole plant
Asparagus racemosus	Asparagaceae	Root
Panax ginseng	Araliaceae	Root
Nelumbo nucifera	Nymphaeaceae	Rhizome and seed
Azadiracta indica	Meliaceae	Leaf
Arnica montena	Compositae	Dried flower head
Calendula officinalis	Asteraceae	Flower
Echinacea purpurea	Asteraceae	Flowering top
Euphorbia tirucalli	Euphorbiaceae	Latex

Ocimum sanctum	Lamiaceae	Leaf

(Source: Kumar et al., 2011)

1.4 Botanical profile of *Hoslundia Opposita*

Its Genus name honors Guinea's Olaus Hoslund-Smith, a young naturalist, from Guinea, who died of fever on his way to Aquapim, in Western Africa according to the Plants Africa website. The specie name, "Opposita" describes the formation of the leaves and fruits (see figure 3), which present in opposite pairs (Pooley, 1998).

1.4.1 Plant Taxonomy

Common names; orange bird berry, bird gooseberry (Engish); “Uyaweyawe” (Zulu); (Codd, 1985) “Oke Ota” (Igbo) “efirin odan” (Yoruba) (Iwu, 1993)

Classification

Kingdom: *Plantae*

Phylum: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Lamiales*

Family: *Lamiaceae*

Genus: *Hoslundia*

Epithet: *opposita* Vahl

Synonyms: *Hoslundia oppositifolia* P. Beauv., *Hoslundia verticillata* Vahl

opposita => with leaves, fruits or other organs inserted on a stem opposite to each other (see figure 3)

verticillata => whorled, three or more leaves springing from the same point (Arbonnier, 2004).

Characteristics

Climate: tropical

Habitat: mesophytic

Habit: shrub; Flower colour: green, beige, white (Arbonnier, 2004).

Figure 3: ***Hoslundia opposita*** **in its natural habitat**

(Source: Pooley, E. 1998)

Figure 4: Top and lower side of *Hoslundia opposita* leaf

(Source: Pooley, E. 1998)

1.4.2 Description of Plant

H. opposita is a herbaceous perennial (either spreading or erect) and sometimes soft shrub, growing up to 1.2 m high. Leaves are opposite or sometimes arranged in threes (figure 3). They are elliptic with serrate margin and short petiole as in figure 4 (Pickering and Roe, 2009). Plants possess minute, white or creamy green-coloured flowers, starting from October to February (Pooley, 1998). Fruits are fleshy, berry-like in shape, tasty, edible and attractively orange-red in colour. It thrives in full sun and well-drained soil. It does best with ample room all around so that it can spread out gracefully (Pooley, 1998).

1.4.3 Geographical Distribution of Plant

Orange bird-berry plants have widespread natural distribution, occurring both in tropical and subtropical open woodland (Codd, 1985; Morton, 1981). In southern Africa they occur naturally in areas such as Namibia and Botswana in the north, as well as in Swaziland. In South Africa, they can be found growing naturally from the

coastal areas of KwaZulu-Natal, extending to Mpumalanga and Limpopo (Pooley, 1998). Plants are very common throughout tropical Africa, in countries such as Senegal, Sudan and Ethiopia. In Nigeria, it originated in Oyo state; and is predominant in south West (Pooley, 1998).

1.4.4 Ecology

Certain insects including bees visit the plant, the tiny cream-green flowers are much loved by butterflies. The fruits are birds' favorites; hence the name orange bird-berry. Wild animals feed on the plant too. *H. opposita* grows along with *Ocimum gratissimum* (scent leaves)*, Ageratum conyzoides* and rabbits feed on them (Codd, 1985). Also, it drives away rodents by the reason of the strong odor it releases being a member of the mint family; thus making it almost impossible for the animals to go into extinction

1.4.5 Cultivation of *H. opposita*

This herbaceous perennial is a hardy garden plant in southern African gardens, but it might not prove hardy in colder climates. Plants require well-drained soil, and perform well in full sun. To encourage hemagglutination growth, plants need to be cut back at least once every year. Propagation is easily obtained from seeds or stem cuttings (Codd, 1985).

1.4.6 Ethnomedicinal uses

In Africa, various parts of the plant are popular remedies for gonorrhea, cystitis, cough, wounds, sores, conjunctivitis, epilepsy, chest pain, stomach disorders, mental disorders and also in management of snake bites (Ayensu and De Filipps,

1978; Watt and Breyer-Brandwijk, 1962). It is also used to treat fever, liver disease, rheumatism and convulsion. The aerial parts are known to be used to treat gonorrhea, cystitis, hookworm, cough, wounds and bilharzias (Chhabra and Uiso, 1994; Ngadjui et al., 1991; Hedberg et al., 1983). Infusion of its leaves is widely used in traditional medicine as a purgative, diuretic, febrifuge, antibacterial and antiseptic. In North western Tanzania, the leaves are mixed with the leaves of *Ocimum basilicum*, boiled and the decoction drunk for treatment of indigestion (Moshi et al., 2009). It is used to treat anaemia in Northern and South- Eastern Cote d'Ivoire (Kone et al., 2012).

1.4.7 Other Uses

People eat the tasty fruits. Leaves are reported to have a strong unpleasant scent, which is alleged to repel bees and is thus utilized in the collection of honey (Pooley, 1998). They can be used successfully to line an informal shrub border or driveways; however, enough space must be left to allow plants to spread comfortably.

1.5 Literature review

It is documented that methanol extract, methanol and ethyl acetate fraction of *H. opposita* stems have hepatoprotective effects against carbon tetrachloride and paracetamol induced liver damage (Akah and Odo, 2010). The crude extract of the entire plant have been found to exhibit strong antibacterial activity and volatile constituents have been identified as well as Jacarandic acid which was also isolated (Ogura et al., 1977; Takahashi et al., 1974). A study had reported that leaves of this plant could be potentially used in the treatment of epilepsy and convulsions (Risa et

al., 2004). Extract from the plant exhibited central nervous system depressant effect (Jide et al., 1999) and acaricidal effect against *Amblyomma variegatum* (Annan et al., 2011).

It is also documented that leaf essential oil of *H. opposita* posses antidyslipidemic effect (Akolade et al., 2011). Pharmacological evaluation of the extract in rodents showed anti inflammatory, analgesic and antipyretic property (Oladije et al., 1998).

1.6 Aim and Scope of Study

I caught an interest on this plant when in 2009 I was told that it is used to treat boils in Nnobi Community of Idemili South Local Government Area of Anambra State. This study was aimed at investigating the immunomodulatory activity of leaves of *H opposita*. The study involved an assay of the crude extract and solvent fractions using delayed type hypersensitivity assay, humoral antibody titre and *in vivo* leucocyte mobilization.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Animals

Adult albino rats (100-150 g) of either sex were used. The animals were obtained from the Laboratory Animal facilities of the Departments of Pharmacology and

Toxicology, and Veterinary Parasitology and Entomology, of University of Nigeria, Nsukka. Animals were housed in steel cages within the facility under standard conditions and allowed free access to commercial pelletized rodent diet (Vital feed Nigeria, Ltd) and water *ad libitum* throughout the study.

2.1.2 Drugs

Levamisole (Reals Pharmaceutical Ltd, Nigeria), Dexamethasone (Pharmacare Ltd, Nigeria), carrageenan (Sigma-Aldrich, Germany).

2.1.3 Chemicals, Solvents and Reagents

(i) Extraction and fractionation

Analytical grades of methanol (Sigma-Aldrich, Germany), n-hexane (Sigma-Aldrich, Germany), ethyl acetate (Sigma-Aldrich, Germany), Silica gel of size 60-120 mesh (Sigma-Aldrich, Germany).

(ii) Pharmacological studies

Carrageenan, Tween 80 (Sigma-Aldrich, Germany), distilled water, normal saline, Phosphate buffer saline, Ethylenediaminetetraacetic acid (EDTA)

(iii) Phytochemical Analysis

Ferric chloride, iodide solution, ethanol, dilute ammonia solution, sulphuric acid, naphthol solution in ethanol, potassium mercuric iodide solution (Mayer's reagent), bismuth potassium iodide solution (Dragendorff's reagent), Fehling's solution, Million's reagent, picric acid solution.

2.1.4 Equipment

Rotary vacuum evaporator (Buchi, Switzerland), oven, petri dishes, spatula, glass chromatographic column, stop clock, filter paper, electronic weighing balance (G&G Electronic Scale, China), animal weighing balance, test tubes, mortar and pestle, beakers, flat bottom conical flask, Thin Layer Chromatography plates (Sigma-Aldrich, Germany), chromatographic tank, pipette, fume chamber.

2.2 Methods

2.2.1 Collection, Identification and Preparation of Plant Material

Fresh leaves of *Hoslundia opposita* Vahl were collected in September 2011 from bushes in Aregbe Obantoko in Abeokuta, Ogun State, Nigeria. The plant was identified and authenticated by O.A. Ugbogu, and O.S. Shasanya, taxonomists at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. It is designated voucher specimen No. 109563. The leaves were cleaned, dried under a shade for 3 days and pulverized to coarse powder using a milling machine.

2.2.2 Extraction of Plant Material

The leaf powder (4.608 kg) was extracted by cold maceration in methanol for 48 h and filtered. The residue was macerated again for 24 h to ensure exhaustive extraction. The residue was repeatedly washed with fresh solvent until filtrate became clear. The filtrate was concentrated using a rotary vacuum evaporator (40°C) under reduced pressure to obtain methanol extract (ME).

2.2.3 Fractionation of ME

The dry extract (174.538 g) was mixed with silica gel by triturating in a mortar to obtain a uniform mix. The mixture was air-dried at room temperature, packed into the glass column and was successively eluted with n-hexane, ethyl acetate and

methanol in order of increasing polarity. The fractions were collected and concentrated in a rotary evaporator (40°C) under reduced pressure to obtain n-hexane fraction (NHF), ethyl acetate fraction (EF) and methanol fraction (MF).

2.2.4 Determination of yield (%)

The yield of extract and fractions were calculated using the relation:

$$\text{yield of extract (\%)} = \frac{\text{weight of extract (g)}}{\text{weight of plant material macerated (g)}} \text{ X } 100$$

$$\text{yield of fraction (\%)} = \frac{\text{weight of fraction(g)}}{\text{weight of extract fractionated(g)}} \text{ X } 100$$

2.2.5 Phytochemical analysis of extract and fractions

The extract and fractions were subjected to phytochemical analysis for identification of constituents using the methods of Harborne (1984).

2.2.5.1 Test for saponins

To 0.25 g of the plant extract (i.e. ME) in 100 ml beaker, about 20 ml distilled water was added and boiled gently in a hot water bath for 2 min. The mixture was filtered while hot and allowed to cool and the filtrate was diluted with 20 ml of water and shaken vigorously and observed for the presence of a stable froth upon standing.

2.2.5.2 Test for tannins

Ferric chloride test

About 1.0 g of extract was boiled with 50 ml of water, filtered and used for the ferric chloride test. To 3 ml of each extract filtrate, few drops of ferric chloride were added and the colour of the resulting precipitate observed. A greenish black precipitate indicates the presence of tannins.

2.2.5.3 Test for resins

Precipitation test

The alcohol extract obtained by extracting about 0.20 g of each extract with 15 ml of 96% ethanol was poured into 20 ml of distilled water in a beaker. A precipitate occurring indicates the presence of resins.

2.2.5.4 Test for flavonoids

Ammonium Test

About 10 ml of ethyl acetate was added to 2 ml of extract and heated on a water bath for 3 min. the mixture was cooled, filtered and filtrate subjected to ammonium test thus: about 4 ml of each filtrate was shaken with 1 ml of dilute ammonium solution. The sugars were allowed to separate and the yellow colour in the ammoniacal layer indicates the presence of flavonoids.

2.2.5.5 Test for steroids and terpenoids

A mixture of about 9 ml of ethanol and 1ml of the plant extract was concentrated to 2.5 ml on a boiling water bath and 5 ml of hot water was added. The mixture was allowed to stand for one hour and the waxy matter filtered off. The filtrate was further extracted with 2.5 ml of chloroform using separating funnel. To about 0.5 ml of chloroform extract in a test tube was added carefully 1 ml of concentrated sulphuric acid to form a lower layer. A reddish brown interface shows presence of

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

2.2.5.6 Test for alkaloids

About 20 ml of 5% sulphuric acid in 50% ethanol was added to about 2 g of plant extract and heated on a boiling water bath for 10 min., cooled and filtered. About 2 ml of the filtrate was treated with a few drops of Mayer's reagent, Dragendorff's reagent, Wagner's reagent and picric acid (1%) solution. The remaining filtrate in about 100 ml separating funnel was made alkaline with dilute ammonia solution, the aqueous alkaline solution was separated and extracted with two 5 ml portion of dilute sulphuric acid. The extract was tested with a few drops of Mayer's, Wagner's, and Draggendorf's reagents and picric acid solution. Alkaloids give milky precipitate with one drop of Mayer's reagent, reddish brown precipitate with one drop of Wagner's reagent, yellow precipitate with one drop of picric acid solution and brick red precipitate with one drop of Dragendorf's reagent.

2.2.5.7 Test for glycosides

Modified Borntrager's test

To about 2 ml of each filtrate was added about 5 ml of dilute sulphuric acid and ferric chloride solution, boiled for 5 min, cooled and filtered into a 50 ml separating funnel. The filtrate was shaken with an equal volume of carbon tetrachloride and the lower organic layer carefully separated into a test tube. About 5 ml of dilute ammonia solution was added to the test tube containing each filtrate and then

shaken. A rose pink to red colour in the ammoniacal layer indicates the presence of anthraquinone glycosides.

2.2.5.8 Test for oil

About 0.1 ml of extract was dropped on filter paper and observed. Translucency of the paper indicates presence of oil.

2.2.5.9 Test for carbohydrates

Iodine test

A drop of iodine solution was mixed with about 0.5 ml of extract. A blue-black colour indicates the presence of starch.

2.2.5.10 Test for reducing sugars

Fehling's test

The filtrate obtained after shaking about 1 ml of extract vigorously with about 5 ml of distilled water, was used in the fehling's test as follows: to about 1 ml portion of the filtrate were added equal volumes of Fehling's solution A and B boiled on water bath for a few minutes. A brick red precipitate indicates the presence of reducing sugar.

All the tests for phytochemical analysis were also carried out for the different fractions.

2.3 Pharmacological studies

2.3.1 Acute Toxicity (LD_{50}) of ME.

The acute toxicity of ME was estimated in mice using the Lorke's method (Lorke, 1983). The tests involved two phases. The first phase is determination of the toxic

range. The mice were randomly placed in three groups (n=3) to receive oral administration of ME (10, 100, 1000 mg/kg) solubilized in of 3%v/v Tween 80. The treated mice were observed for 24 h for number of deaths.

The doses for the second phase were determined by death pattern in the first phase. There was no death recorded in the first phase, thus a fresh batch of four mice received 1000, 1600, 2900, or 5000 mg/kg of ME respectively. They were observed for lethality or signs of acute intoxication for 24 h. The LD_{50} is the geometric mean of the highest nonlethal dose and the least lethal dose (Lorke, 1983).

2.3.2 Carrageenan Induced Leucocyte Mobilization in Rats.

The rats of both sexes were randomly alloted (n=5) to six groups. Groups 1, 2, and 3 received the extract ME (200, 400 or 800 mg/kg). Group 4 received levamisole (2.5 mg/kg) for positive control and group 5 received 3% Tween 80 (2.5 ml/kg) as negative control. Administration was by the oral route according to their body weight. After one hour, each rat received intraperitoneal injection of 0.5 ml of 1% (w/v) carrageenan suspension in normal saline. Four hours later, the rats were sacrificed and the peritoneum washed with 5 ml of 5% solution of EDTA in Phosphate Buffered Saline (PBS) to recover the peritoneal fluid (Ribeiro et al., 1991). Total and differential leukocyte counts in the peritoneal fluid were performed.

This experimental protocol was carried out for HF, EF, and MF (200, 400 and 800 mg/kg).

2.3.3 Sheep Red Blood Cell (SRBC)-Induced Delayed Type Hypersensitivity Assay

Delayed type hypersensitivity was induced in rats using SRBCs as antigen (Sharma, 1994). Five groups (n=5) of rats were used for this test. The first, second and third groups received 200, 400 or 800 mg/kg of the extract respectively. Group 4 received 2.5 mg/kg of levamisole while group five received 2.5 ml/kg of vehicle (3% Tween 80) as negative control. All drug administration was done orally. One hour later, each animal received 0.1 ml of 40% sheep red blood cell (SRBC) suspension injected into the subplantar region of the right hind paw. The day was taken as day zero. Drug administration was continued once daily for seven days. On the 7^{th} day, the sizes of the left hind paw of the rats were measured by water displacement and the animals challenged by injecting 0.1 ml of 40% SRBC into the left subplantar of the paw (Doberty, 1981). The volume of the paws of each rat was measured 24 h after the challenge.

This experiment was also carried out for each of the fractions.

2.3.4 Hemagglutination Antibody Titre in rats

Five groups (n=5) of rats were used for this test. The first, second and third groups received 200, 400, 800 mg/kg of the extract respectively. Group 4 received 2.5 mg/kg of levamisole while group five received 2.5 ml/kg of vehicle (3% Tween 80) as negative control. All drug administration was done orally. One hour later, each animal received 0.1 ml of 40% sheep red blood cell (SRBC) suspension injected into the subplantar of the right hind paw. The day was taken as day zero. Drug administration was continued once daily for seven days. On the 7^{th} day, blood samples were collected from the retro-orbital plexus and serum separated to estimate primary hemagglutination antibody titre (Sharma et al., 1994).

Two fold diluted serum in saline (25 µl) was challenged with 25 µl of 1% (v/v) SRBC in U-shaped microtitre plates and incubated at 37°C for 1 hour and then observed for hemagglutination. The highest dilution giving visible hemagglutination was taken as primary antibody titre.

The animals were challenged on day 7 by injecting 0.1 ml of 40% SRBC into the subplantar of the paw after collecting blood samples from the retro-orbital plexus. Drug administration was continued for the next seven days. On day fourteen, blood samples were collected for secondary antibody titre (Sharma et al., 1994).

2.4 Statistical analysis

Results were expressed as Mean ± standard error of mean (SEM), n=5. The results obtained were analyzed using SPSS (version 16.0) and subjected to least significant difference (LSD) post-hoc test. Differences between means of treated and control groups were accepted significant at $P<0.05$.

CHAPTER THREE

RESULTS

3.1 Extraction and fractionation

The extraction yielded 194.324 g of the methanol extract (ME; 4.22% W/W). Fractionation of 174.538 g of ME yielded 23.8 g of n-hexane (NHF; 13.636% w/w), 50.787 g of ethyl acetate (EF; 29.09%w/w), and 74.02 g of methanol (MF; 42.41%w/w) fractions respectively (Table 3).

3.2 Phytochemical constituents of extract and fractions

The ME gave positive reactions for alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids, reducing sugar, resins, tannins, protein, and fats and oils. The NHF tested positive to resins, steroids, terpenoids, and fats and oils. The EF tested positive to flavonoids, alkaloids, and resins while MF gave positive reactions for alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids, reducing sugar, tannins, protein, and fats and oils (Table 4).

3.3 Acute toxicity (LD_{50}) of ME

The ME (5000 mg/kg) administered orally did not cause lethality and signs of acute intoxication after 24 h observation period. The LD_{50} is therefore indeterminate (Table 5).

Table 3: Yield (%) of extract and fractions

Extract	Yield (%w/w)
ME	4.22
NHF	13.636
EF	29.09
MF	42.41

Table 4: Phytochemical constituents of extract and fractions

Phytochemical Constituent	Relative Presence			
	ME	NHF	EF	MF
Flavonoids	+	-	++	+
Alkaloids	++	-	+	++
Glycosides	++	-	-	++++
Resins	+++	+++	++	-
Steroids	+	+++	-	+
Terpenoids	+++	++++	-	+
Fats & Oils	++	++++	-	+
Saponins	++	-	-	+++
Tannins	+	-	-	++
Reducing Sugar	+	-	-	+

Protein	++	-	-	++
Acidic compounds	-	-	-	-

Key

- = Absent

+ = Present in small concentration

++ = Present in moderately high concentration

+++ = Present in very high concentration

++++ = Abundantly Present

3.4 Pharmacological studies

3.4.1 Effect of ME and fractions on *in vivo* leucocyte mobilization in rats

The ME elicited a significant and non-dose related increase in Total Leucocyte Count (TLC). It increased neutrophils and elicited mild decrease in lymphocyte count (Table 6). The NHF, EF and MF elicited dose dependent and significant increase in TLC. They also increased neutrophils but decreased lymphocytes with the exception of EF (200 & 800 mg) which elicited mild reduction in neutrophil count with mild increase in lymphocyte count (Tables 7).

Table 5: Acute Toxicity (LD_{50}) of ME

Dose of extract (mg/kg)	Mortality
10	0
100	0
1000	0
1600	0
2900	0
5000	0

Table 6: Effect of ME on *in vivo* leucocyte mobilization in rats

Treatment	**Dose (mg/kg)**	**TLC (cells/mm^3)**	**Differential Leucocyte Count (%)**		
			Neutrophils		**Lymphocytes**
			Monocytes		
ME	200	9900±1456.7* (83.3)	11.4±0.6 {8.1}	88.6±0.6 (1.4)	0.0±0.0
	400	7880±526.68 (45.9)	16.6±2.44 (33.9)	83.2±2.48 {4.8}	0.2±0.2
	800	7060±2322.8	15.6±3.46	84.4±3.46	0.0±0.0

		(30.7)	(25.8)	{3.4}	
Levamisole	2.5	6960±1034.7 (28.8)	14.4±3.26 (16.1)	85.6±3.26 {2.1}	0.0±0.0
Control	2.5 ml/kg	5400±1244.9	12.4±1.78	87.4±1.75	0.2±0.2

n=5 per group; *P<0.05 compared to control LSD post-hoc

Values in parenthesis represent percentage increase (%) in Total Leucocyte Count, Neutrophils or Lymphocytes as the case may be compared to control while values in curly bracket represent percentage decrease {%} in Neutrophils or Lymphocytes as the case may be compared to control.

Table 7: Effect of NHF, EF and MF on *in vivo* leucocyte mobilization in rats

Treatment	Dose (mg/kg)	TLC (cells/mm^3)	Differential Leucocyte Count (%)		
			Neutrophils	Monocytes	Lymphocytes
NHF	200	21800±2219.9* (79.6)	9.2±1.59 (21.1)	90.8±1.59 {1.7}	0.0±0.0
	400	28400±3942.9* (133.9)	10.6±1.60 (39.5)	89.2±1.59 {3.5}	0.2±0.2
	800	29000±2717.3* (138.8)	9.4±3.08 (23.7)	90.6±3.08 {1.9}	0.0±0.0
EF	200	27400±1426.5* (125.7)	7.4±1.08 {2.6}	92.6±1.08 (0.22)	0.0±0.0
	400	30800±5096.5* (153.7)	11±3.36 (44.7)	89±3.36 {3.7}	0.0±0.0
	800	48900±641.87* (302.8)	6.4±1.17 {15.8}	93.4±1.21 (1.1)	0.2±0.2
MF	200	11400±322.49	10.6±2.16	89±2.12	0.4±0.4

		{6.1}	(39.5)	{3.7}	
	400	29400±965.40*	8±0.71	91.2±1.07	0.8±0.8
		(142.2)	(5.3)	{1.3}	
	800	34700±694.26*	13±2	87±2	0.0±0.0
		(185.8)	(71.1)	{5.8}	
Levamisole	2.5	12900±1184.9	12.2±2.82	87.4±2.82	0.4±0.4
		(6.3)	(60.5)	{5.4}	
Control	2.5 ml/kg	12140±2640	7.6±0.93	92.4±0.93	0.0±0.0

n=5 per group; *P <0.05 compared to control LSD Post hoc; Values in parenthesis represent percentage increase (%) in Total Leucocyte Count, Neutrophils or Lymphocytes as the case may be compared to control while values in curly bracket represent percentage decrease {%} in Total Leucocyte Count, Neutrophils or Lymphocytes as the case may be compared to control.

3.4.2 Effect of ME and fractions on Delayed type hypersensitivity reaction (DTHR) in rats

400 and 800 mg/kg of methanol extract elicited a significant (P<0.05) and non-dose related inhibition of delayed type hypersensitivity response in rats but not 200 mg/kg ME (Table 8). The fractions (200, 400 and 800 mg/kg) elicited a significant (P<0.05) and dose related inhibition of DTHR (Table 9).

3.4.3 Effect of ME and fractions on hemagglutination antibody titre in rats

The ME elicited a dose dependent and significant (P<0.05) elevation of primary antibody titre compared to the control. The secondary antibody titre increased with increase in dose of MF; however it was lower than the control (Table 10). Rats treated with the fractions produced a lower primary and secondary antibody titre

than in the control group; however antibody titre slightly increased with higher doses of the fractions (Table 11).

Table 8: Effect of ME on delayed type hypersensitivity reaction in rats

Treatment	Dose (mg/kg)	Edema (ml)	Inhibition of edema (%)
ME	200	0.3±0.02*	85
	400	0.05±0.02*	75
	800	0.03±0.02*	85
Levamisole	2.5	0.13±0.02*	33
Control	-	0.2±0.3	-

n=5 per group; *$P < 0.05$ compared to control LSD Post hoc

Table 9: Effect of NHF, EF, and MF on Delayed type hypersensitivity reaction in rats

Treatment	Dose (mg/kg)	Edema (ml)	Inhibition of edema(%)

NHF	200	0.163±0.03*	37.79
	400	0.113±0.02*	56.87
	800	0.05±0.00*	80.92
EF	200	0.21±0.03	19.85
	400	0.1±0.03*	61.83
	800	0.09±0.01	65.65
MF	200	0.11±0.02*	58.02
	400	0.09±0.03*	65.65
	800	0.082±0.03*	68.70
Levamisole	2.5	0.056±0.02	78.63
Control	-	0.262±0.04	-

n=5 per group; *P <0.05 compared to control LSD Post hoc

Table 10: Effect of ME on hemagglutination antibody titre in rats

Treatment	**Dose (mg/kg)**	**Hemagglutination Antibody Response**	
		Primary	Secondary
ME	200	4±0.32	3±0.55*
	400	5.67±0.18*	4.67±0.48
	800	6.33±0.48*	6.0±0.63
Levamisole	2.5	5.67±0.18*	6.0±0.63

Control	2.5 ml/kg	4.33±0.48	6.33±0.85

n = 5 per group; * $P < 0.05$ compared to control LSD Post hoc

Table 11: Effect of NHF, EF and MF on hemagglutination antibody response in rats

Treatment	**Dose (mg/kg)**	**Hemagglutination Antibody Response**	
		Primary	Secondary
NHF	200	3.75±0.73*	7.75±1.16*
	400	4.0±0.32	8.2±0.49
	800	4.0±0.32	9.5±0.22
EF	200	4.8±0.20	7.8±0.58*
	400	3.4±0.40*	8.0±0.63*

	800	4.0±0.32	9.5±0.22
MF	200	4.0±0.32	7.6±0.51*
	400	4.2±0.49	8.4±0.51
	800	3.8±0.58	8.4±0.68
Levamisole	2.5	3.6±0.87*	6.6±0.75*
Control	2.5	5.25±0.73	10±0.00

n = 5 per group; * $P < 0.05$ compared to control LSD Post hoc

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 Discussion

This experiment investigated the immunomodulatory effect of methanol extract and fractions of *H. opposita* leaves in rats. Methanol extract and fractions elicited increase in Total Leucocyte Count and neutrophil count compared with the negative

control. The differential count showed that neutrophils were the most mobilized leucocyte. The increase in neutrophil count compared to the control group may help in increasing the general resistance of the body (as PMNs are the primary cells which engulf and eliminate invading micro-organisms) against microbial infections via phagocytosis (Wheater and Stevens, 2002). It has been observed that the chemotactic movement of neutrophils towards the foreign body is the first and most important step in phagocytosis (Ganachari et al., 2004). Little percentage of monocytes was mobilized while eosinophils and basophils were absent. Monocytes float in the blood stream, enter tissue and turn into macrophages which clean up pus as part of the healing process. They share the phagocytic function of neutrophils and also present pieces of pathogens to T cells so the pathogens may be recognized again and killed.

The manifestation of DTHR induced by sheep red blood cell was inhibited by the ME and the fractions. Decrease in DTHR revealed the inhibitory effect of methanol extract and fractions on T lymphocytes required for the expression of the reaction (Vinothapooshan and Sundar, 2011). DTHR has been shown to be absolutely dependent on the presence of memory T cell (both CD4+ and CD8+) which also produce interferon-gamma (IFN-ɤ)-producing $CD4^+$ (THI) or $CD8^+$ (TCI) T cells (Biedermann et al., 2001; Allen, 1999; Furr, 1998). Also, in 1940, Chase and Landsteiner proved that DTHR was mediated by the cellular not the humoral arm of the immune system. It usually takes 24-72 h to develop and it involves activation of T-cells which results in the infiltration of monocytes and lymphocytes into the area of inflammation because the immune cells are also involved in mediating

inflammatory responses. The observed inhibition of DTHR may be related to the anti-inflammatory property of this plant (Iwu and Igboko, 1982), as immune cells are also involved. This inhibition can occur by immune deviation which entails steering T-cells towards an IL-4 producing TH2 or TC2 phenotypes (Biedermann et al., 2001). Levamisole acts by elevating cGMP levels in lymphocytes. Its imidazole ring seems to be one of the active moieties responsible for the functional increase of peripheral T-cell and macrophages (Alan et al., 2001). Thus levamisole should not inhibit DTHR.

Administration of ME caused a clear reduction in secondary antibody titre compared with the control but primary antibody titre at doses of 400 and 800 mg/kg were higher than the control. Primary and secondary antibody titres in the fraction treated rats were lower than the control. The secondary antibody titres of all animals except the ME treated rats were expectedly higher than the primary, since subsequent antigenic stimulation of primary-sensitized animals may result in high antibody production as there is now an expanded clone of cells with memory of the original antigen available to proliferate into mature plasma cells (Furr, 1998). This result was expected since very little monocytes were mobilized. Sasaki and others have observed that depletion of monocytes from peripheral blood mononuclear cells of patient with systemic lupus erythematosus resulted in a decreased antibody synthesis *in vitro* and addition of monocytes restored the response by lymphocytes (Sasaki et al, 1989). It is not immediately clear why the secondary titre in ME treated rats were lower than primary titre.

Humoral immunity is the aspect of immunity that is mediated by secreted antibodies. Antibody synthesis requires the cooperation of at least 3 major cell types; the macrophages, B lymphocytes and T lymphocytes (Benecerraf, 1978; Janeway et al., 2001) but the cells most ostensibly involved in antibody production are the B lymphocytes (Lauralee, 2004). These cells make antibodies if stimulated to do so, but they are incapable of stimulating themselves. The primary response consists mainly of immunoglobin M, whereas the secondary response consists mainly of immunoglobin G (Ratt et al., 2001). The decrease in antibody titre is consistent with the mild decrease in lymphocyte count produced by extract and fractions. This result may indicate that the methanol leaf extract and fractions of *Hoslundia opposita* has immunomodulatory effects on the cell mediated component of the immune system.

Phytochemical analysis of the extract and fractions revealed the presence of tannins, flavonoids, sterols/terpenoids, resins, saponins, alkaloids, reducing sugar, acidic compounds, cardiac glycosides and proteins. It has been observed that immunomodulatory substances from natural sources could play a role in disease prevention and treatment, especially with the increasing global emphasis on natural system and the campaign on “greening” of the society. So there has been a growing interest in identifying and characterizing natural compounds with immunomodulatory activity (Wang et al., 1991) ever since their possible uses in medicine have been suggested. They include compounds such as polysaccharides, phenols, alkaloids (Engels et al., 1992; Ingolfsdottir et al., 1994). The various phytochemical constituents detected are known to exhibit medicinal activity as well

as physiological activity (Sofowora, 1993). Flavonoid reduces cancer by interfering with enzyme that produces estrogen (Farquer, 1996). Bioflavonoids have been demonstrated to possess anti-oxidant properties which help combat free radical oxidative stress. It also has protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatotoxins, viruses, and tumours (Barakat et al., 1993; Okwu and Omodamiro, 2005). They serve as defence mechanisms against predation by many micro-organisms, insects and herbivores (De et al., 1999). Tannins have been traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, hemorrhoids and diarrhea (Ogunleye and Ibitoye, 2003). Flavonoids exhibit antibacterial activity.

The results above suggest that the immunomodulatory effect of *H opposita* may be found in tannins, glycosides, flavonoids, alkaloids, steroids, and terpenoids. Previous studies implicated these phytoconstituents in the immunomodulatory activity of some plants. For example, alkaloid, tannins, flavonoids, glycosides and sterols from *Adhatoda vasica* Linn (Viothapooshan and Sundar, 2011); tannins from *Gymnema sylvestre* (Trease and Evans, 1993; Gupta et al., 2010); terpenoids, sterols, proteins and tannins from *Azadirachta indica* (Nat et al., 1987), *Terminalia chebula* (Sohni and Batt, 1996), and *Lawsonia alba* (Kulkarni and Karande, 1998) are considered to exhibit immunomodulatory property (Biswas et al., 2002).

Acute toxicity study of *H. opposita* in mice estimated the oral LD_{50} to be greater than 5000 mg/kg; thus *H. opposita* is safe (Lorke, 1983) and implies a remote risk of possible acute intoxication.

The results of this study reveal that the extract and fractions of *Hoslundia opposita* have immunomodulatory effect. These results suggest that *Hoslundia opposita* has effect on cell-mediated components of the immune system, although the immunomodulatory activity is yet to be associated with the specific constituents of the leaves.

4.2 Conclusion

The results of this study have established immunomodulatory activity of *Hoslundia opposita* extract and fractions and justified the claims made by herbalists about the use of its leaf to treat abscess, catarrh and wounds.

Further studies are recommended to identify and isolate the exact constituent(s) responsible for the immunomodulatory effect and also establish the mechanism of action.

REFERENCE

Ahsan,N., Hricik, D., Matas, A., Rose, S., Tomlanovich, S., Wilkinson, A., Ewell, M., Mclntosh,M., Stablein, D., Hodge, E. (1999). Prednisone withdrawal in kidney transplant recipients on cyclosporine and mycophenolate mophetil-a prospective randomized study. Steroid withdrawal study group. *Transplantation,* 68: 1865–1874.

Akah, P.A., Odo, C.I. (2010). Hepatoprotective effects of the solvent fractions of the stem of *Hoslundia opposita* Vahl. (Lamiaceae) against Carbon tetrachloride and paracetamol-induced liver damage in rats. *International Journal of Green Pharmacy,* 4(1): 54–58.

Akbar, A.N., Terry, L., Timms, A., Beverly, P.C., Janossy, G. (1988). Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells: *Journal of Immunology,* 140(7): 2171–2178.

Akolade, J.O., Muhammed, N.O., Usman, L.3., Owolarafe, T.A. Oloyede, O.B. (2011). Antidyslipidemic effect of leaf essential oil of *Hoslundia opposita* Vahl. In Allosan-induced diabetic rats. *International Journal of Tropical Medicine and Public Health,* 1(1): 1–29.

Alan, M.K., Terry, B.S., Jeffrey, A.B. (2001). Immunomodulators: immunosuppressive agents, tolerogens and immunostimulants. *Goodman and Gilman's The pharmacological basis of therapeutics*; 10th ed. United States: McGrawHill Companies.

Alberts, B., Alexander J., Julian L., Martin R., Keith R., Peter W. (2002). *Molecular Biology of the Cell.* (4th Edition). New York and London: Garland Science.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Water, P. (2002). *Molecular biology of the cell*. Garland science. New York. P. 1367.

Alder, M.N., Rogozin, I.B., Lyer, L.M/. Glazko, G.V., Cooper, M.D., Pancer, Z. (2005). Diversity and function of Adaptive Immune receptors in Jawless vertebrate.

Allen, B. (1999). Delayed type hypersensitivity: current theories with an historic perspective. *Dermatology Online Journal* 5(1): 7.

Al-Razi (2003). The Columbia Electronic Encyclopedia, 6th ed. Columbia University press.

Amirghofran, Z., Azadbakht, M., Karimi, M.H. (2000). Evaluation of the immunomodulatory effects of five herbal plants. *Journal of Ethnopharmacology,* 72(1-2): 167–172.

Anderson, N., Nester, R. (2007). *Microbiology: A human perspective*. (5th Ed.)

Andry, N. (1700). Germ theory calendar. 3rd ed. London. Rhodes and Bells printers.

Annan, K., Jackson, N., Dickson, R.A., Sam, G.H., Komlaga, G. (2011). Acaricidal effect of an isolate from *Hoslundia opposita* vahl against *Amblyomma variegatum*. *Pharmacognosy Research*, 3(3): 185-188.

Arbonnier, M. (2004). *Trees, shrubs and lianas of West African dry zones*. CIRAD; Paris.

Auphan, N., Didonato, J.A., Rosette, C., Helmberg, A., Karin, M. (1995). Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of 1 kappa B synthesis. *Science*, 270: 286–290.

Aw, D., Silva, A.B., Palmer, D.B. (2007). Immunosenescence: emerging challenges for an ageing population. *Immunology* 120(4): 435–446.

Ayensu, E.S., De Filipps, R.A. (1978). Endangered and threatened plants of the United States. Washington, D.C: Smithsonian Institution.

Babu T.D., Kuttan G., Padikkala J. (1995). Cytotoxic and anti-tumour properties of certain texa of Umbelliferae with special reference to *Centella asiatica*. *Journal of Ethnopharmacolology,* 48: 53–57.

Barakat, M.Z., Shahab, S.K., Darwin, N., Zahemy, E.L. (1993). Determination of ascorbic acid from plants. *Anal Biochemistry*, 52, 225–245.

Baron, S., Coppenhaver, D.H., Dianzani, F. (1992) Introduction to the interferon system. In: Baron, S., Dianzani, F., Stanton, G.J., Fleischmann, W.R., Jr. Coppenhaver, D.H., Hughes, T.K., Klimpel, G.R., Niesel, D.W., Tyring, S.K. (Eds.). *Interferon: Principles and Medicinal Applications* .Galveston, Texas, Pp. 1-15.

Benacerraf, R.A. (1978). Hypothesis to relate the specificity of T lymphocytes and the activity of I region Ir genes in macrophages and B lymphocytes. *Journal of Immunology,* 120: 1809–1812.

Bergstresser, P.R., Sullivan, S., Streilein, J.W., Tigelaar, R.E. (1985). Origin and function of Thy-1+ dendritic epidermal cells in mice. *Journal of Investigative Dermatology,* 85(1): 855–905.

Bertino, J.R. (1973). Chemical action and pharmacology of methotrexate, azathioprine and cyclophosphamide in man. *Arthritis & Rheumatology,* 16:79–83.

Biedermann, T.R., Maailhammer, A., Mai, C., Sander, A., Ogilivie, F., Brombacher, K., Maier, D., Rocken, M.L. (2001). Reversal of established delayed type hypersensitivity reactions following therapy with IL-4 or antigen specific Th2 cells. *Euopean Journal of Immunology*, 5: 1582–1591

Biswas , K., Chattopadhya, I., Banerjee, R. K., Banfyopadhyay U. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*) *Current Science*, 82(11): 1136–1345.

Black, C.A. (1999). Delayed type hypersensitivity: Current theories with an historic perspective. *Dermatology Online Journal,* 5(1): 7.

Bourdage, J.S., Hamlin, D.M. (1995). Comparative polyclonal antithymocyte globulin and antilymphocyte/antilymphoblast globulin anti-CD antigen analysis by flow cytometry. *Transplantation*, 59: 1194–1200.

Brown, P. (2001). Cinderella goes to the ball. *Nature,* 410: 1018-1020.

Bukowski, R.M. (1999). Immunotherapy in renal cell carcinoma. *Oncology (huntington*), 13: 801–810.

Burke, J.F. Jr., Pirsch, J.D., Ramos, E.L., Salomon, D.R., Stablein, D.M., Van Buren, D.H., West, J.C. (1994). Long-term efficacy and safety of cyclosporine in renal-transplant recipients. *New England Journal of Medicine*, 331: 358–363.

Cao, J., Park, I., Cooper, A., Sodroski, J. (1996). Molecular determinants of acute single-cell lysis by human immunodeficiency virus type 1. *Journal of Virology,* 70**:** 1340–1354.

Chabner, B.A., Ryan, D.P., Paz-AresL., Garcia-CarboneroR., Lalabnesi, P. (2001). Antineoplastic agents. *Goodman and Gilman's The pharmacological basis of therapeutics.* 10th ed. united states: McGrawhill, New York, p. 1395.

Chan, G.L., Canafax, D.M., Johnson, C.A. (1987). The therapeutic use of Azathioprine in renal transplantation. *Pharmacotherapy*, 7:165–177.

Chandra, R.K. (1997). Nutrition and the immune system: an induction. *The American Journal of Clinical Nutrition* 66(2): 4605–4635.

Chase, M.W., Landsteiner, K. (1940). Studies on the sensitization of animals with simple chemical compounds: VII. *Journal of Experimental Medicine,* 71(2): 237-245.

Chhabra, S.C., Uiso, F.C. (1994). Antibacterial activity of some Tanzanian plants used in traditional medicine. *Fitoterapia*, 62(6): pp 499–503

Codd, L.E. (1985). Lamiaceae. Flora of southern Africa. Botanical research Institute, Pretoria. Pp. 284.

Coico, R., Sunshine, G., Benjamin, E. (2003). *Immunology: A short Course*. Garland publishing New York. 5th ed. p. 48

Copeland, K.F., Heeney, J.L. (1996). T helper cell activation and human retroviral pathogenesis.

De M., Krishina De A., Baneriee A.B. (1999). Antimicrobial screening of some Indian species. *Phytother . Res.* pp 616–618.

Diasio, R.B., LoBuglio, A.F. (1996). Immunomodulators: Immunosuppressive agents and Immunostimulants. . In: Hardman J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W. Goodman, G.A. (Eds.). *Goodman and Gilman's The pharmacological basis of therapeutics*. 9th ed. McGraw-Hill, New York, pp 1291 – 1308.

Doberty, N.S. (1981). Selective effect of immunosuppressive agents against the delayed hypersensitivity response and humoral response to sheep red blood cells in mice. *Agents Actions,* 11:237–242.

Duff, G.W. (1985). Interleukin-1 and fever production. *Rheumatology,* 24(1)12–14.

Engels, F., Renirie, B.F., 'tHart, B.A., Labadie, R.P., Nikamp, F.P. (1992). Effect of apocynin, a drug isolated from roots of Picrorhiza kurroa on arachidonic acid metabolism. *FEBS Letters,* 305, 245–256.

Farnsworth N.R., Bunyapraphatsara N. (1992). *Thai medicinal plants: recommended for primary health care systems*. Pp 305.

Farquer, J.N. (1996). Plant sterols. Their Biological effects in humans. Handbook of lipids in human nutrition. BOCA. Paton FL CRC Press, pp: 101–105.

Faulds, D., Goa, K.L., Benfield, P. (1993). Cyclosporine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. *Drugs*, 45: 278–298.

Figueredo, A., Germond, C., Maroun, J., Brownman, G., Walker-Dilks, C., Wong, S. (1997). Adjuvant therapy for stage II colon cancer after complete resection. *Provincial Gastrointestinal Disease Site Group. Cancer Prevention Control*, 1: 379–392.

Firestein, G.S. (2007). The inflammatory response. In: Goldman L, Ausiello, D. eds. Cecil medicine. 23rd ed. Philadelphia, PG: saunders Elsevier; chap. 45.

Fuchimoto, Y., Yamada, K., Shimizu, A., Yasumoto, A., Sawada, T., Huang, C.H., Sachs, D.H. (1999). Relationship between chimerism and tolerance in a kidney transplantation model. *Journal of Immunology*, 162: 5704–5711.

Furr, S.R. (1998). Fundamentals of Immunobiology. In: *Pharmaceutical Microbiology*. Hugo, W.B., Russell, A.D. (Ed.), 6th Edn., Oxford: Blackwell Science Ltd., pp. 278-303.

Gaffney, K., Scot D.G. (1998). Azathioprine and cyclophosphamide in the treatment of rheumatoid arthritis. *British Journal of Rheumatology,* 37:824–836.

Gaffney, K., Scott, D.G. (1998). Azathioprine and cyclophosphamide in the treatment of rheumatoid arthritis. *British Journal of Rheumatology*, 37: 824–836.

Ganachari, M.S., Kumar, S., Bhat, K.G. (2004). Effect of ziziphus jujuba leaves extract on phagocytosis by human neutrophils. *Journal of Natural Redmedies,* 4: 47–51.

Gell, P.G.H., Coombs, R.R.A. (1963). *Clinical aspects of immunology*, 1st ed. Oxford, England: Blackwell.

Ghaffar, A. (2006). Hypersensitivity reactions. *Immunology.* Bookmark Printers. chap. 17

Gherardi, E. (from Answers.com, 2006). The concept of immunity, history and applications. Immunology course Medical school, University of Pavia. *The Columbia Electronic Encyclopedia*, 6th ed. Columbia University Press.

Glushakova, S., Grivel, J.C., Fitzgerald, W., Sylwester, A., Zimmerberg, J., Margolis, L.B. (1998). Evidence for the HIV-1 phenotype switch as a causal factor in acquired immunodeficiency. *Nature Medicine,* 4**:** 346–349.

Goronzy, J.J., Weyand, C.M. (2007). The innate and adaptive immune systems. In: Goldman L., Ausiello, D. eds. Cecil medicine. 23 ed. Philadelphia, Pa: Saunders Elsevier; chap. 42.

Gowans, J.L., McGregor, D.D., Cowen, D.M. (1962). Initiation of immune responses by small lynmphocytes. *Nature,* 196: 651–655.

Greenberg, S. (1957). A concise history of immunology: The role of smallpox in the development of vaccination. Chap. 5.

Grivel, J.C., Margolis. (1999). CCR5- and CXCR4-tropic HIV-1 are equally cytopathic for their T-cell targets in human lymphoid tissue. *Nature Medicine,* 5**:** 592–593.

Guermonprez, P., Valladeau, J., Zituogel, L., Theyr, C., Amigorena, S. (2002). Antigen presentation and T cells stimulation by dendritic cells. *Annual review of Immunology*, 20(1): 621–67.

Guo, Y., Lee, C., So, K.H., Gao, J., Yeung, W.S.B., Yao, Y., Lee, K. (2013). Human Leukocyte Antigen-Gs activates extracellular signal-regulated protein kinase signaling and stimulates trophoblast invasion. DOI: 10.1371/Journal. Pone.0076023.

Gupta, B., Pramanik, S., Tiwari, O.P., Thacker, N., Pande, M., Upmanyu, N. (2010). Immunomodulatory activity of Gymnema Sylvestre leaves. *The internet Journal of Pharmacology*, 8(2): 10.

Hale, D.A., Gottschalk, R., Umemura, A., Maki, T., Monaco, A.P. (2000). Establishment of stable multilineage hematopoietic chimerism and donor-specific tolerance without irradiation. *Transplantation*, 69: 1242–1251.

Hanabuchi, S., Ito, T., Park, W.R., Watanabe, N., Shaw, J.C., Roman, E., Arima, K., Wang, Y.H., Voo, K.S., Lao, W., Liu Y.J. (2010). Thymic stromal lymphopoietin-activated plasmacytoid dendritic cells induce the generation of FOXP3+ regulator T cells in human tumour. *Journal of Immunology,* 184161: 2999–3007.

Harborne, J.B.C (1984). *Phytochemical methods: a guide to modern technique of plant analysis*. (2nd Ed.). Chapman and Hall Ltd., London. Pp.282.

Hasket, R.F. (1985). Diagnostic categorization of psychiatric disturbance in Cushing's syndrome. *American Journal Psychiatry*, 142: 911-916.

Hayden, F.G. (2001). Antimicrobial agents continued, Antiviral agents (Non reetroviral) In: Hardman J.G., Limbird, L.E. (Eds.), *Goodman and Gilman's The pharmacological basis of therapeutics*. 10th ed. New York: McGraw-Hill, p 1346.

Haynes, B.F., Markert, M.L., Sempowski, G.D., Patel, D.D., Hale, L.P. (2000). The role of the thymus in immune reconstitution in aging, bone marrow transplantation and HIV-1 infection. *Annual Review of Immunology,* 18: 529–560.

Hedberg, I., Hedberg, O., Madati, P.J., Mshigeni, K.E., Mshiu, E.N., Samuelson, G. (1983). Inventory of plants used in traditional medicine in Tanzania. *Journal of Ethnopharmacol*ogy, 9(1): 105–127.

Herbein, G., Van Lint, C., Lovett, J.L., Verdin, E. (1998). Distinct mechanisms trigger apoptosis in human immunodeficiency virus type 1-infected and in uninfected bystander T lymphocytes. *Journal of Virology,* 72**:** 660–670.

Hillman, S.H. (2001). Hematopoietic agents, growth factors, minerals and vitamins. *Goodman and Gilman's The pharmacological basis of therapeutics.* 10th ed. McGrawhill, New York, p. 1494.

Hirose, R., Roberts, J.P., Quan, D., Osorio, R.W., Freise, C., Ascher, N.L., Stock, P.G. (2000). Experience with daclizumab in liver transplantation: renal transplant dosing without calcineurin inhibitors is insufficient to prevent acute rejection in liver transplantation. *Transplantation*, 69: 307–311.

Hoffmeister, R.T. (1983). Methotrexate therapy in rheumatoid arthritis: 15 years experience. *American Journal Medicine*, 75:69-73.

Holm, G.H., Zhang, C., Gorry, P.R., Peden, K., Schols, D., De Clercq, E., Gabuzda, D. (2004). Apoptosis of Bystander T cells induced by human immunodeficiency virus Type 1 with increased envelope/receptor affinity and co receptor binding site exposure. *Journal of Virology*, 78(9): 4541-4551

Holtmeier, W., Kabelitz, D. (2005). Gamma delta T cells link innate and adaptive immune responses. *Chemical immunology and allergy* 86: 151–183.

Hong, J.C., Kahan, B.D. (1999). Use of anti-CD25 monoclonal antibody in combination with rapamycinto eliminate cyclosporine treatment during the induction phase of immunosuppression. *Transplantation*, 68: 701–704.

Hong, J.C., Kahan, B.D. (2000). Immunosuppressive agents in organ transplantation: past, present and future. *Seminars in Nephrolology*, 20: 108–125.

Hunt, R., Mitzi, N. (2004). *Microbiology and Immunology online*. University of South Carolina.

Ingolfsdottir, K., Jureie, K., Fischer, B., Wagner, H. (1994). Immunomodulatory active polysaccharide from Cetraria islandica. *Planta Medica* 60, 527–531.

Inohara, I., McDonald, C., Nuñez, G. (2005). "NOD-LRR proteins: role in host-microbial interactions and inflammatory disease". *Annual Review of Biochemistry,* 74: 355–383.

Intayot Y., Kinouchi T., Kataoka K. (2002). Antimutagenicity of Murdannia loriformis in the Salmonella mutation assay and its inhibitory effects on azoxymethane-induced DNA methylation and aberrant crypt focus formation in male F344 rats. *Journal of investigative Medicine,* 49: 25–34.

Iwasaki, A., Medzhitov, R. (2010). Regulation of adaptive immunity by the innate immune system. *Science* 327: 291–295.

Iwu , M.M., Igboko, A.O. (1982). The flavonoids of Garcinia kola. *Journal of Natural Products*, 45: 650–651.

Iwu, M.M. (1993). *Handbook of African Medicinal plants*. Boca Raton, Florida: CRC Press. P. 192.

Jacobson, J.M., Greenspan, J.S., Spritzler, J., Ketter, N., Fahey, J.L., Vasquez, G.J., Whol, D.A. (1997). Thalidomide for the treatment of oral aphthous ulcers in patients with human immunodeficiency virus infection. National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group. *New England Journal of Medicine,* 336: 1487–1493.

Janeway C. A., (2005). *Immunobiology.* (6th ed.). Garland Science.

Janeway, C., Travers, P., Waalport, M., Shlomchik, M. (2001). *Immunobiology*: 5th ed. New York and London Garland Science. Chap. 1 & 2.

Jekle, A., Keppler, O.T., De Clercq, E., Schols, D., Weinstein, M., Goldsmith, M.A. (2003). In vivo evolution of human immunodeficiency virus type 1 toward increased pathogenicity through CXCR4-mediated killing of uninfected CD4 T cells. *Journal of Virology,* 77**:**5846–5854.

Jerner, E. (1955). The natural selection theory of antibody formation. *Proceedings of the Natural Academy of Sciences,* 41: 849–857.

Jiang, H., Chess L. (2004). An integrated viewof suppressor T cell subsets in immunoregulation. *Journal of clinical Investigation,* 114(9): 1198–1208.

Jide,O.A., Awe, S.O., Makinde, J.M. (1999). *Journal of Phytotherapy Research,* 13(5): 425–426.

Johnson, H.M., Bazer, F.W., Szente, B.E., jarpe, M.A. (1994). How interferon fight disease. *Scientific American*, 270: 68–75.

Joos, L., Tamm, M. (2005). Breakdown of pulmonary host defence in the immunocompromised host: cancer chemotherapy. *Proceedings of the American Thoracic society* 2(5): 445–448.

Kahan, B.D., Julian, B.A., Pescovitz, M.D., Vanrenterghem, Y., Neylan, J. (1999a). Sirolimus reduces the incidence of acute rejection episodes despite lower cyclosporine doses in Caucasian recipients of mismatched primary renal allografts: a phase II trial. Rapamune Study Group. *Transplantation*, 68: 1526–1532.

Kahan, B.D., Rajagopalan, P.R., Hall, M. (1999b). Reduction of the occurrence of acute cellular rejection among renal allograft recipients treated with basiliximab, a

chimeric anti-interleukin-2-receptor monoclonal antibody. United States Simulect Renal Study Group. *Transplantation*, 67: 276–284.

Kaiser, G.E. (2009). Interleukin-1 and fever. *Microbiology*, 11(5): 780–795.

Kapoor, R., Viijan, V., Singh, P. (2008). Bacillus Calmette Guerin in the management of superficial bladder cancer. *Indian Journal of Urology*, 24(1): 72-76.

Katja, H., Arnette, B., Elena, C., Simone, R., Ulrich, G., Werner, S., Friedrich, G., Ulf, S. (2002). Wegener's granulomatosus: antiproteinase 3 antibodies induce monocyte, cytokine and prostanoid release- role of autocrine cell activation. *Journal of Leukocyte Biology,* 71: 996–1004.

Kendall, M. (2007). Fine airborne urban particles (pm 2.5) sequester lung surfactant and amino acids from human lung lavage. *Journal of physiology,* 293(4): 1053–8.

Khazan, W., M'bika, J.P., Tomkiewicz, C. (2005). Expression of macrophage-selective markers in human and rodent adipocytes. *FEBS Lett*ers, 579(25): 5631–5634.

Khoury, S., Sayegh, M.H., Turka, L.A. (1999). Blocking costimulatory signals to induce transplantation tolerance and prevent autoimmune disease. *International Reviews of Immunology*, 18: 185–199.

Kiessling, R., Klier, E., Pross, H., Wigzell, H. (1975). Natural killller cells in the mouse II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cells. *European Journal of Immunology,* 5: 117–121.

Kimball, J.A., Pescovitz, M.D., Book, B.K., Norman, D.J. (1995). Reduced human IgG anti-ATGAM antibody formation in renal transplant recipients receiving mycophenolate mofetil. *Transplantation*, 60:1379–1383.

Kirk, A.D., Harlan, D.M., Armstrong, N.N., Davis, T.A., Dong, Y., Gray, G.S., Hong, X., Thomas, D., Fechner, J.H. Jr., Knechtle, S.J. (1997). CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proceedings of the Natural Academy of Sciences,* 94: 8789–8794.

Kofi, A., Nora, J., Rita, A.D., George, H., Sam, I., Gustav, K. (2011). Acaricidal effect of an isolate from *Hoslundia opposita* vahl against Amblyomma variegatum (Acari: Ixodidae). *Pharmacognosy Research,* 3(3): 185–188.

Kone, W.M., Koffi, A.G., Bomisso, E.L.,Tra Bi F.H. (2012). Ethnomedical study and Iron content of some medicinal herbs used in traditional medicine in Cote D'Ivoire for the treatment of anaemia. *African Journal of traditional, complementary and alternative medicine*, 9(1).

Kovarik, J.M., Kahan, B.D., Rajagopalan, P.R., Bennett, W., Mulloy, L.L., Gerbeau, C., Hall, M.L. (1999). Population pharmacokinetics and exposure-response relationships for basiliximab in kidney transplantation. *Transplantation*, 68: 1288–1294.

Kreis, H., Cisterne, J.M., Land, W., Wramner, L., Squifflet, J.P., Abramowicz, D., Campistol, J.M., Morales, J.M., Grinyo, J.M., Mourad, G., Berthoux, F.C., Brattstrom, C., Lebranchu, Y., Vialtel, P. (2000). Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. *Transplantation*, 69: 1252–1260.

Krensky A.M., Strom T.B., Bluestone, J.A. (2001). Immunomodulators: Immunosuppressive agents, Tolerogens, and Immunostimulants. In*, Goodman and Gilman's, The Pharmacological Basis of Therapeutics*, 10th ed. McGraw Hill, New York. pp. 1463–1484.

Krensky, A.M., Weiss, A., Crabtree, G., Davis, M.M., Parham, P. (1990). T-lympphocyte-antigen interactions in transplant rejection. *New England Journal of Medicine*, 322: 510–517.

Krishnaswamy, G., Ajitawi, O., Chi, D.S. (2006). The human mast cell: an overview. *Methods in molecular biology* 315: 13–34.

Krombach, F., Munzing, S., Allmeling, A.M., Gerlach, J.T., Behr, J., Dooger, M. (1997). Cell size of alveolar macrophages: an interspecies comparison. *Environmental Health perspectives,* 105(5): 1261–3.

Kulkarni, S.R., Karande, V.S. (1998). *Journal of Indian drugs*. 35(7). Pp 427–433.

Kumar, S.V., Kumar, S.P., Rupesh, D., Nitin, K. (2011). Immunomodulatory effects of some traditional medicinal plants. *Journal of Chemical and Pharmaceutical Research*, 3(1): 675-684.

Kumar, S.V., Kumar, S.P., Rupesh, D., Nitin, K. (2011). Immunomodulatory effects of some traditional medicinal plants. *Journal of Chemical and Pharmaceutical Research,* 3(1): 675-684.

Kuo, C.J., Chung, J., Fiorentino, D.F., Flanagan, W.M., Blenis, J., Crabtree, G.R. (1992). Rapamycin selectively inhibits interleukin -2 activation of p70S6 kinase. *Nature,* 358:70–73.

Kuo, C.J., Chung,J., Fiorentino, D.F., Flanagan, W.M., Blenis, J., Crabtree, G.R.(1992). Rapamycin selectively inhibits interleukin-2 activation of p70 S6 kinase. *Nature*, 358: 70–73.

Lane, N.E., Lukert, B. (1998). The science and therapy of glucocorticoids induced bone loss. *Endocrinology Metabolism Clinics of North America*, 27: 465-483.

Larsen, C.P., Alexander, D.Z., Hollenbaugh, D., Elwood, E.T., Ritghie, S.C., Aruffo, A., Hendrix, R., Pearson, T.C. (1996). CD40-gp39 interactions play a critical role during allograft rejection. Suppression of allograft rejection by blockade of the CD40-gp39 pathway. *Transplantation*, 61:4–9.

Lary, J.M., Daniel, K.L., Erickson, J.D., Roberts, H.E., Moore, C.A. (1999). The return of thalidomide: can birth defects be prevented? *Drug safety*. 21:161–169.

Lauralee, S. (2004). *Human physiology: From cells to systems*. 7th ed. London: West publishing company. Pp 450–461.

Levin, R.E., Kalidas, S., Gopinadhan, P., Pometto, A. (2006). *Food biotechnology,* 2nd ed. CRC press, USA. P 1028.

Lindquester , G.J. (2006). Introduction to the history of disease. Disease and Immunity. USA: Rhodes College. Chap. 1–3.

Lo Iacono, O., castro, A., Diago, M., Moreno, J.A., Fernandez-bermejo, M., Vega, P., Garcia-Buey, L., Garcia-monzon, C., Pedreira, J., Moreno-Otero, R. (2000). Interferon alfa-2b plus ribavirin for chronic hepatitis C patients who have not responded to interferon monotherapy. *Ailment*ary *Pharmacology & Therapeutics*, 14: 463–469.

Lo,T.N., Almeida, A.P., Beaven, M.A. (1982). Dextran and carrageenan evoke different inflammatory responses in rats with respect to composition of infiltrates and effect of indomethacin. *Journal of pharmacology and experimental therapeutics*, 221:261–267.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology,* 53: 275.

Lu, Y., Koga, Y., Tanaka, K., Sasaki, M., Kimura, G., Nomoto, K.(1994). Apoptosis induced in $CD4^+$ cells expressing gp160 of human immunodeficiency virus type 1. *Journal of Virology,* 68**:** 390–399.

Madigan, M., Martinko, J. (2005). *Biology of microorganisms*. 11th ed. USA: Prentice Hall. Chap. 7.

Male, D., Brostoff, J., Roth, D., Roitt, I. (2006). *Immunology*. 7th ed. pp 19–28.

Mansour, S.M. (2008). Invariant Natural Killer T cells in Immune Surveillance and Tumor Immunotherapy: Perspectives and Potentials. *Archives of Iranian Medicine*. 11(2): 186–195.

Mariat, C., Alamartine, E., Diab, N., de Filippis. J.P., Laurent, B., Berthoux, F.A. (1998). A randomized prospective study comparing low-dose OKT3 to low-dose ATG for the treatment of acute steroid –resistant rejection episodes in kidney transplant recipients. *Transplant International*, 11: 231–236.

Mayer, A.D., Dmitrewski, J., Squifflet, J.P., Besse, T., Grabensee, B., Klein, B., Eigler, F.W., Heemann, U., Pichlmayr, R., Behrend, M., Vanrenterghem, Y., Donck, J., Van Hooff, J., Christiaans, M., Morales, J.M., Andres,A., Johnson, R.W., Short, C., Buchholz, B., Rehmert, N., land, W., Scleibner, S., Forsythe, J.L., Talbot, D., Neumayer, H.-H., Hauser, I., Ericzon, B.-G., Brattstrom, C., Claesson, K., Muhlbacher, F., Pohanka, E. (1997). Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: a report of the European Tacrolimus Multicenter Renal Study Group. *Transplantation*, 64: 436–443.

McCune, J.M. (2001). The dynamics of $CD4^+$ T-cell depletion in HIV disease. *Nature*, 410**:**974–979.

Medzhitov, R., Janeway, C. (2000). Innate Immunity. Outstanding clear coverage of the mechanisms involved in innate immunity and its significance for the adaptive immune response *New England Journal of Medicine,* 343: 338-344.

Miller, J.F. (1993). Self-non self discrimination and tolerance in T and B lymphocytes. *Immunologic research* 12(2): 115–130.

Miller, J.F. (2002). The discovery of thymus derived lymphocytes. *Immunological Reviews* 185(1): 7–14.

Miller, M.T., Stromland, K. (1991). Teratogen update: thalidomide: a review, with a focus on ocular findings and new potential uses. *Teratology*, 60: 306–321.

Mitchell, R.S., Kumar, V., Abbas, A.K., Fausto, N. (2007). *Robbins basic pathology*. 8th ed. Philadeiphia: Saunders. Pp 431–436.

Moertel, C.G., Flemming, T.R., Macdonald, J.S., Haller, D.G., Laurie, J.A., Goodman, P.J., Ungerleider, J.S., Emerson, W.A., Tourney, D.C., Glick, J.H., Veeder, M.H., Mailliard, J.A. (1990). Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. *New England Journal of Medicine*, 322: 352–358.

Morales, A., Ottenhof, P., Emerson, L. (1981). Treatment of residual, non-infiltrating bladder cancer with bacillus Calmette-Gueri., *Journal of Urology,* 125: 649–651.

Morton, J.F. (1981). *Atlas of medicinal plants of middle America: Bahamas to Yucatan Sprigfield.* Illinois, USA, pp. 745–750.

Moshi, M.J., Otieno, D.F., Mbabazi, P.K., Weisheit, A. (2009). The ethnomedicine of the Haya people of Bugabo ward, Kagera region, North western, Tanzania. *Journal of ethnobiology and ethnomedicine*, 5: 24.

Murphy, B., Krensky, A.M. (1999). HLA-derived peptides as novel immunomodulatory therapeutics. *Journal of the American Society of Nephrology*, 10: 1346–1355.

Nat, V.D., Klerx, J.P., Dijk, V.H., Desilva, K.T. (1987). *Journal of Ethnopharmacology*, 19. Pp 125–131.

Natsumeda, Y., Carr, S.F. (1993). Human type 1 and 11 IMP dehydrogenases as drug targets. *Annals of the New York Academy of Science*, 696: 88–93.

Netea, M.G.,Van der G.C., Van der Meer J.W.M., Kullberg, B. (2004). The role of Toll-like receptors in host defense: bringing specificity to the innate immune response. *Journal of Leukocyte Biology*, 75: 749–755.

Ngadjui, B.T., Ayafor, J.F., Sondengam, B.L., Connolly, D.J., Rycroft, D., Tillequim, F. (1991). Phytochemistry 32(5). 1313-1315. Hoslundin, hoslundal, and hoslunddiol: three new flavonoids from the twigs of *Hoslundia opposita* (Lamiaceae). *Tetrahedron*, 47, 3555–3564.

Niedzwiecki, A., Rasnick, D., Mhlongo, S., Ratt, M.R.D. (2005). The leading cause of Immune deficiency disease worldwide. *Dr Ratt's cellular health series*.

Ogunleye, D.S., Ibitoye, S.F. (2003). Studies of antimicrobial activity and chemical constituents of Ximenia Americana. *Tropical Journal of Pharmaceutical Research*, 2(2): 239–241.

Ogura, M., Cordell, G.A., Farnsworth, N.R. (1977). Llodia 40: 157

Okwu D., Omodamiro, O.D. (2005). Effect of hexane extract and phytochemical content of Xylopia ethiopia and Ocimum aratissimum on the uterus of guinea pig. *Biological Research*, p 3.

Olajide, O.A., Oladiran, O.O., Awe, S.O., Makinde, J.M. (1998). Pharmacological evaluation of *Hoslundia opposita* extract in rodents. *Pharmacotherapy research*, 12(5). Pp 364–366.

Oldham, R. K. (1983). Natural killer cells: Artifact to reality. *Cancer and Metastasis Reviews*, 2(4): 323–336.

Opelz, G., Terasaki, P.I. (1978). Improvement of kidney-graft survival with increased numbers of blood transfusions. *New England Journal of Medicine,* Med. 229:799-803.

Opelz, G., Vanrenterghem, Y., Kirste, G., Gray, D.W., Horsburgh, T., Lachance, J.G., Largiader, F., Lange, H., Vujaklija-Stipanovic, K., Alvarez-Grande, J., Schott, W., Hoyer, J., Schnuelle, P., Descoeudres, C., Ruder, H., Wujciak, T., Schwarz, V. (1997). Prospective evaluation of pretransplant blood transfusions in cadaver kidney recipients. *Transplantation*, 63: 964–967.

Ortho Multicenter Transplant Group. (1985). A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. *New England Journal of Medicine,* 313: 337–342.

Parham, P. (2005). *The Immune system*. Garland Science, New York.

Patard, J.J., saint, F., Velotti, F., Abbou, C.C., Chopin, D.K. (1998). Immune response following intracesical bacillus Calmette- Guerin instillations in superficial bladder cancer: a review, *Urological Research*, 26:155–159.

Paterson, D.L., Patel, A. (1998). Bacillus Calmete-Guerin (BCG) immunotherapy for bladder cancer: review of complications and their treatment. *Australian and New Zealand Journal of Surgery*, 68: 340–344.

Patwardhan, B., Kalbag, D., Patki P.S., Nagsampadi, B.A. (1990). Search of Immunomodulatory agents: a review. *India drugs*, 28(2): 348–358.

Pickering, H., Roe, E. (2009). *Wild flowers of the Victoria falls Area*. Helen Pickering, London. P. 67.

Pilat, N.I., Hock, K., Wekerle, T. (2012). Mixed cell chimerism through donor bone marrow transplantation: a tolerogenic cell therapy for application in organ transplantation. *Current Opinion in Organ Transplantation*, 17(12): 63-70.

Pooley, E. (1998). *A field guide to wild flowers kwaZulu-Natal and eastern Region*. Natal Flora Publication Trust, Durban.

Priyanka,S., Mansi, V., Jha, K.K., Maanju, P. (2012). *Journal of Advanced Scientific Research*, 3(1): 7–12.

Punt, C.J. (1998). The use of interferon- alpha in the treatment of cutaneous melanoma: a review. *Melanoma Research*, 8:95–104.

Punturee, K., Kasinrerk, W., Wild, C.P., Vinitketkumnen, U. (2005). Immunomodulatory effects of Thai medicinal plants. *Chiang Mai Medbull,* 44(1): 1-12.

Rang, H.P., Dale, M.M., Ritter, J.M., Flower, R.T. (2007). Local Hormones, Inflammation and Immune reactions. In: *Pharmacology*. 6th ed. Elsevier, London. Pp 203–247.

Ransohoff, R.M. (1998). Cellular responses to interferons and other cytokines: the JAK-STAT paradigm. *New England Journal of Medicine*, 338: 616–618.

Ratt, I.M; Brostoff, J.; Male, D.K. (2001). *Immunology*, 6th edition. Elsevir science: Philadelphia, pp. 1–440

Regan, T.V. (2003). *The Gell-Coombs classification of hypersensitivity rections: a re-interpretation*.8th ed. saunders.

Reuter H.D., (1995). Allium sativum and Allium ursinum: part 2. Pharmacology and medicinal application. *Phytomedicine,* 2: 73–91.

Revenis, M.E., Kaliner, M.A. (1992). Lactoferrin and lysozyme deficiency in airway secretions: association with the development of bronchopulmonary dysplasia. *Journal of Pediatrics,* 121 (2): 262-270.

Ribeiro, R.A., Flores, C.A., Cunha F.Q., Ferreira, S.H. (1991). IL-8 causes *in vivo* neutrophil migration by a cell dependent mechanism. *Immunology*, 73: 472–477.

Risa, J., Risa, A., Adsersen, A., Gauguin, B., Stafford, G.I., Van, S.J., Jager, A.K. (2004). Screening of plants ued in southern Africa for epilepsy and convulsions in GABA A-benzodiazepine receptor assay. *Journal of Ethnopharmacology*, 93, 177–182.

Roitt, I., Brostoff, J.., Male, D. (2001). *Immunology* (6th ed.) pp 480.

Rostaing, L., Chabannier, M.H., Modesto, A., Rouzaud, A., Cisterne, J.M., Tkaczuk, J., Durand, D. (1999). Predicting factors of long - term results of OKT3 therapy for steroid resistant acute rejection following cadaveric renal transplantation. *American Journal of Nephrology*, 19: 634–640.

Rothenberg, M., Hogan, S. (2006). The Eosinophils. *Annual Review of Immunology*, 24(1): 147-174.

Sabatino, D.E., Nichols, T.C., Merricks, E., Bellinger, D.A., Herzog, R.W., Monahan P.E. (2012). Annual models of hemophilia; progress in molecular biology and Translational Science 105: 151–209.

Sampaio, E.P., Kaplan, G., Miranda, A., Nery, J.A., Miguel, C.P., Viana, S.M., Sarno, E.N. (1993). The influence of thalidomide on the clinical and immunologic manifestation of erythema noodosum leprosum. *Journal of Infectious Diseases*, 168: 408–414.

Sanchez, L., Calvo, M., Brock, J.H. (1992). *Biological role of lactoferrin Archives of Disease in Childhood*, 67(5): 657-661.

Sasaki, T., Shibata, S., Hirabayashi, Y., Sekiguchi, Y., Yoshinaga, K. (1989). Accessory cell activity of monocytes in anti- DNA antibody production in systemic lupus erythematosus. *Clinical & Experimental Immunology*, 77(1): 37–42.

Schreiber, S.L., Crabtree, G.R (1992). The mechanism of action of cyclosporine A and FK506. *Immunology Today,* 13: 136–142.

Schwarz B.A., Bhandoola, A. (2006). Trafficking from the bone marrow to the thymus: a prerequisite for thymopoiesis. *Immunological Reviews,* 209: 47–57.

Sharma, M.L., Rao, C.S., Duda, P.L. (1994). Immunostimulatory activity of Picrorhiza Kurnoa leaf extract. *Journal of Ethnophamacology,* 41: 85–192.

Silverstein, A.M. (1989). *History of immunology*. London: Academic Press. Chap. 1.

Singh, M., O'Hagan, D. (1999). "Advances in vaccine adjuvants". *Nature Biotechnology,* 17 (11): 1075–81.

Sinkovics, J.G., and Horvath, H.C. (2000). Vaccination against human cancers (review). *International Journal of Oncology*, 29: 81–96.

Smithells, R.W., Newman, C.G. (1992). Recognition of thalidomide defects. *Journal of Medical Genetics*, 29: 716–723.

Sofowora L.A. (1993). *Medicinal plants & traditional medicine in Africa*. Spectrum Books ltd, Ibadan, pp 55–71.

Sohni, Y.R., Bhatt, R. M. (1996). Activity of a crude extract formulation in experimental hepatic amoebiasis and in Immunomodulation studies. *Journal of Ethnopharmacology*, 54: 119–124.

Spitzer, T.R., Delmonico, F., Tolkoff-Rubin, N., McAfee, S., Sackstein, R., Saidman, S., Colby, C., Sykes, M., Sachs, D.H., Cosimi, A.B. (1999). Combined histocompatibility leukocyte antigen-matched donor bone marrow and renal transplantation for multiple myeloma with end stage renal disease: the induction of allograft tolerance through mixed lymphohematopoietic chimerism. *Transplantation*, 68: 480–484.

Sproul, T.W., Cheng, P.C., Oykstra, M.L., Pierce, S.K. (2000). A role for MHC class II antigen processing in B cell development. *International reviews of Immunology,* 19(2-3): 139–155.

Starzl, T.E., Demetris, A.J., Murase, N., Trucco, M., Thomson, A.W., Rao, A.S., Fung, J.J. (1997). Chimerism after organ transplantation. *Current Opinion in Nephrology and Hypertension*, 6: 292–298.

Stevenson, J.R. (2011). *Cells and organs of the immune system*. London: Rutledge. Chap. 2.

Stvrtinova, V., Jan, J., Ivan, H. (1995). *Inflammation and Fever from Pathophysiology: Principles of Disease.* Computing centre, Slovak Academy of science: Academic electronic press.

Suwannasaen, D., Romphruk, A., Leelayuwat, C., Leitmemongkolchai, G. (2010). Bystander T cells in human Immune responses to dengue antigens. *BioMed Central Journals of Immunol*, 20 (11): 47.

Takahashi, K., Kawaguchi, S., Nishimura, K.I., Kubota, K., Tanabe, Y., Takani, M. (1974). *Chemistry pharmaceutical bull*. 22: 650

Takiishi, T., Korf, H., Van Belle, T.L., Robert, S., Gysemans, Conny., Mathieu, C. (2012). Reversal of autoimmune diabetes by restoration of antigen-specific tolerance using genetically modified lactococcus lactis in mice. *Journal of clinical Investigation,* 122(5): 1717-1725.

Tilg, H., Kaser, A. (1999). Interferons and their role in inflammation. *Current Pharmaceutical Design*, 5: 771–785.

Tompkins, W.A. (1999). Immunomodulation and therapeutic effects of the oral use of interferon-alpha: mechanism of action. *Journal of Interferon & Cytokine Research,* 19: 817–828.

Trease, E.G., Evans, W.C. (1993). *Pharmacognosy.* Balliere Tindale, London, pp 278 - 539.

Undem, B.J., Lichtenstein, L.M. (2001). Drugs used in the treatment of asthma. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. (Joel F. Hardman and Lee E. Limbird eds) 10th ed. McGraw Hill, New York. Pp 734–735.

Vincenti, F. (2006). Costimulatory blockade-What will the future bring. *Transplantation*, 22(5): 1293-1296.

Vinitketkumnuen, U., Charoenkunathum W., Kongtawelert, P., Lertprasertsuke, N., Matsushima, T. (1996). Antimutagenicity and DT- diaphorase inducer activity of Thai medicinal plant, *Murdannia loriformis*. *Journal of Herbs, Species and Medicinal plants*, 4: 45–52.

Vinitketkumnuen, U., Puatanachokchai R., Kongtawelert, P., Lertprasertsuke, N., Matsushima, T. (1994). Antimutagenicity of lemon grass (*Cymbopogon citrates* Stapf) to various known mutagens in Salmonella mutation assay. *Mutation Research,* 341: 71–75.

Vinothapooshan, G., Sundar K. (2011). *African journal of Pharmacology,* 5(3), pp 306–310.

Vivier, E., Raulet, D.H., Moretta,A., Caligiuri, M.A., Zitvogel, L., Lanier, L.L., Yokoyama, W.M., Ugolini, S. (2011). Innate or adaptive imunity; the example of Natural killer cells. *Science,* 331:44–49.

Wang, J.Z., Mao, X.J., Ito, H., Shimura, K. (1991). Immunomodulatory activity of polysaccharide from Acanthopanex obovatus roots. *Planta medica,* 57: 335–336.

Warwick, R., Williams, P.L. (1973). *Angiology*. 5th ed. Longman, London. pp. 588–785.

Watanabe, N., Wang, Y.H., Lee, H.K., Ito, T., Wang, Y.H., Cao, W., Lui, Y. (2005) Hassal's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. *Nature,* 436:1181–1185.

Watt, J.M., Breyer-Brandwijk, M.G. (1962). *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. 2nd ed. Livingstone, London.

Weinstein, G.D. (1977). Methotrexate. *Annals of Internal Medicine*, 86: 199-204.

Wheater, P.R., Stevens, A. (2002). *Wheater's Basic Histopathology: a colour actions and text*. Edinburgh: Churchill livingstone.

Whittington, R., Faulds, D. (1993). Interleukin-2. A review of its pharmacological properties and therapeutic use in patients with cancer. *Drugs*, 46:

Willinger, T., Freeman, T., Hasegawa, H., McMichael, A.J., Callan, M.F. (2005). Molecular signatures distinguish human central memory from effector memory CD8T cell subsets. *Journal of Immunology*, 175 (9): 5895-5903.

Winkelhake, J.L., Gauny, S.S. (1990). Human recombinant interleukin-2 as an experimental therapeutic. *Pharmacological Reviews*, 42:1–28.

Woodle, E.S., Xu, D., Zivin, R.A., Auger, J., Charette, J., O'Laughlin, R., Peace, D., Jollife, L.K., Haverty, T., Bluestone, J.A., Thistlewaite, J.R. Jr. (1999). Phase I trial of humanized, Fc receptor non-binding OKT3 antibody, huOKT3gamma 1 (Ala-Ala) in the treatment of acute renal allograft rejection. *Transplantation*, 68: 608–616.

Worboys, M. (2008). *Spreading germs: Disease theories and medical practice in Britain, 1865-1900*. Cambridge: Cambridge University press.

Yang C.S., Wang Z.Y., Hong J.Y. (1994). Inhibition of tumorigenesis by chemical from garlic and teas. In: Jacobs MM (Eds.) *Diet and cancer: marker, prevention and treatment*. Plenum press, New York. Pp. 113–122.

Young, B., Lowe, J.S., Steven, A., Health, J.W. (2006). *Wheater's functional Histology* (5^{th} ed.)

Zen, K., Parkos, C.A. (2003). Leukocyte epithelial interactions. *Current Opinion in Cell Biology*, 15(5): 557–564.

Zoorob, R.J., Cender, D. (1998). A different look at corticosteroids. *American Family Physician*, 58: 443–450.

APPENDIX

Figure A1: Percentage Inhibition of DTH by ME

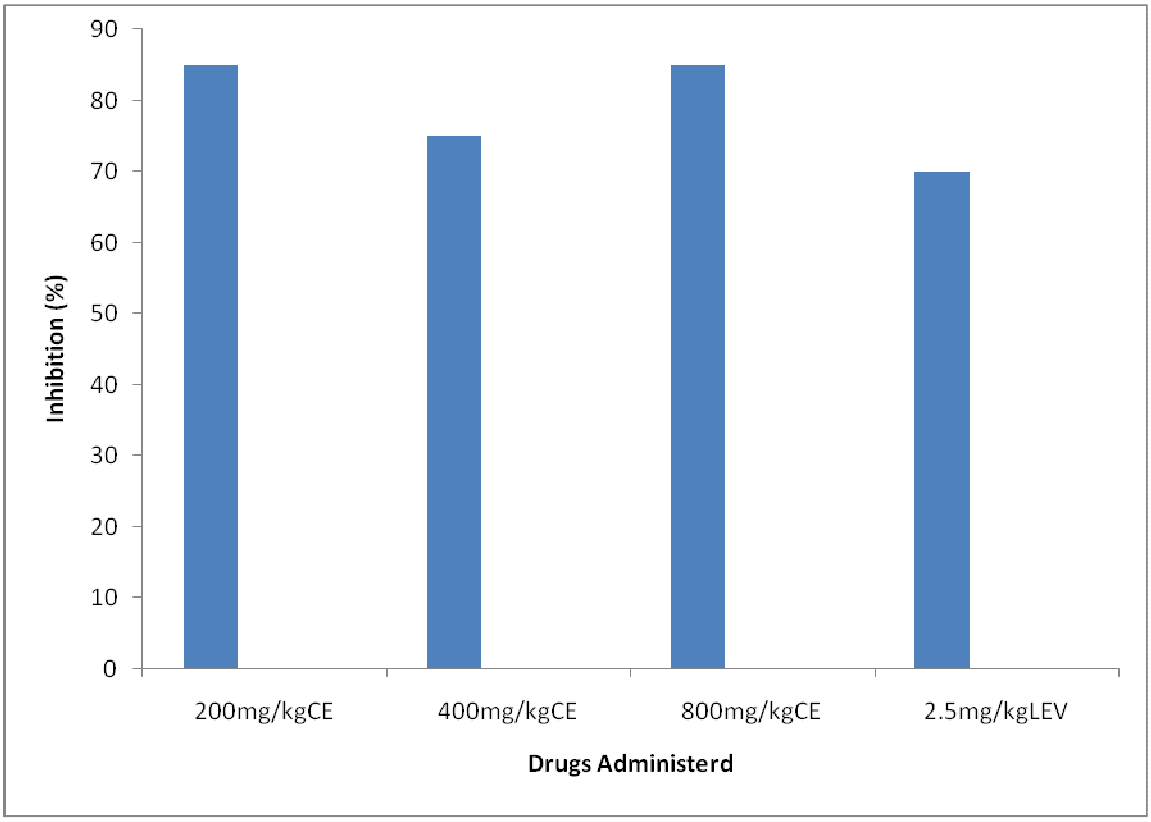


Figure A2: Percentage inhibition of DTH by NHF

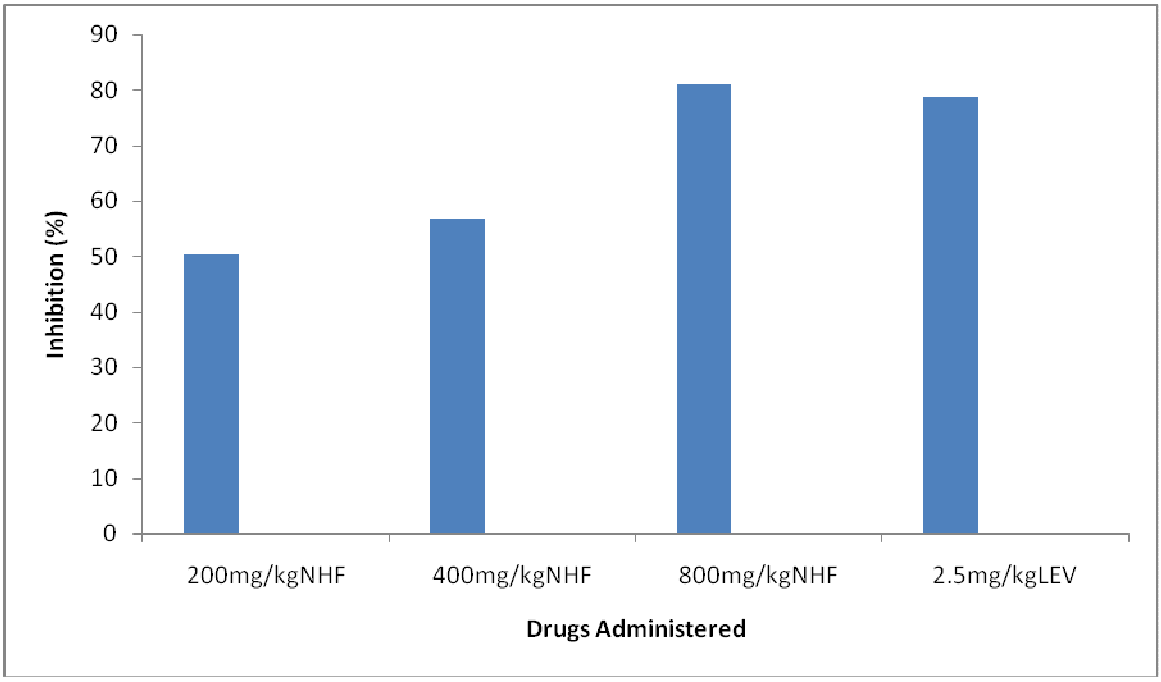


Figure A3: Percentage Inhibition of Delayed Type Hypersensitivity by EF

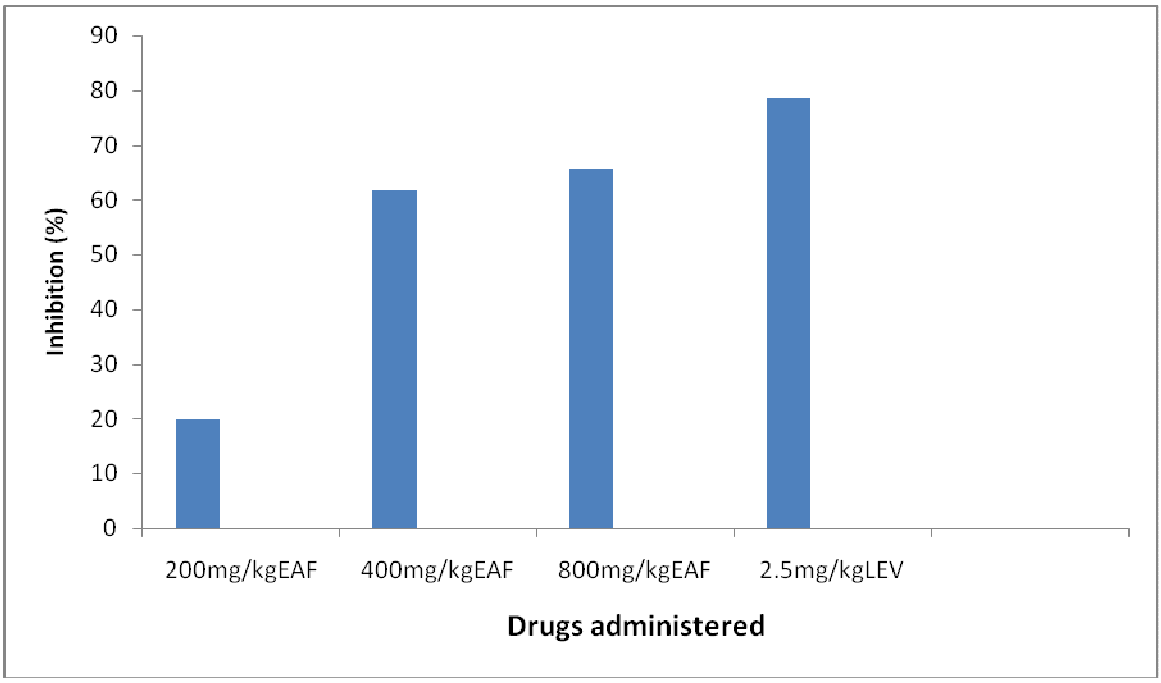


Figure A4: Percentage Inhibition of Delayed Type Hypersensitivity by MF

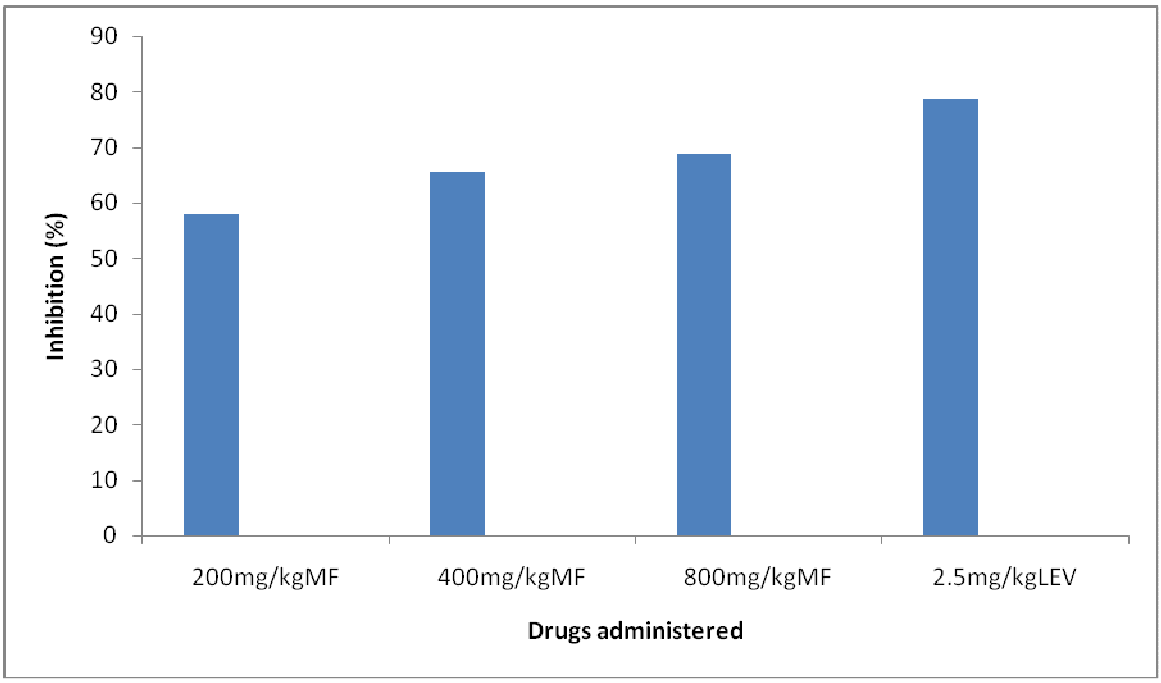


Figure A5: Percentage Inhibition of Delayed Type Hypersensitivity by the fractions

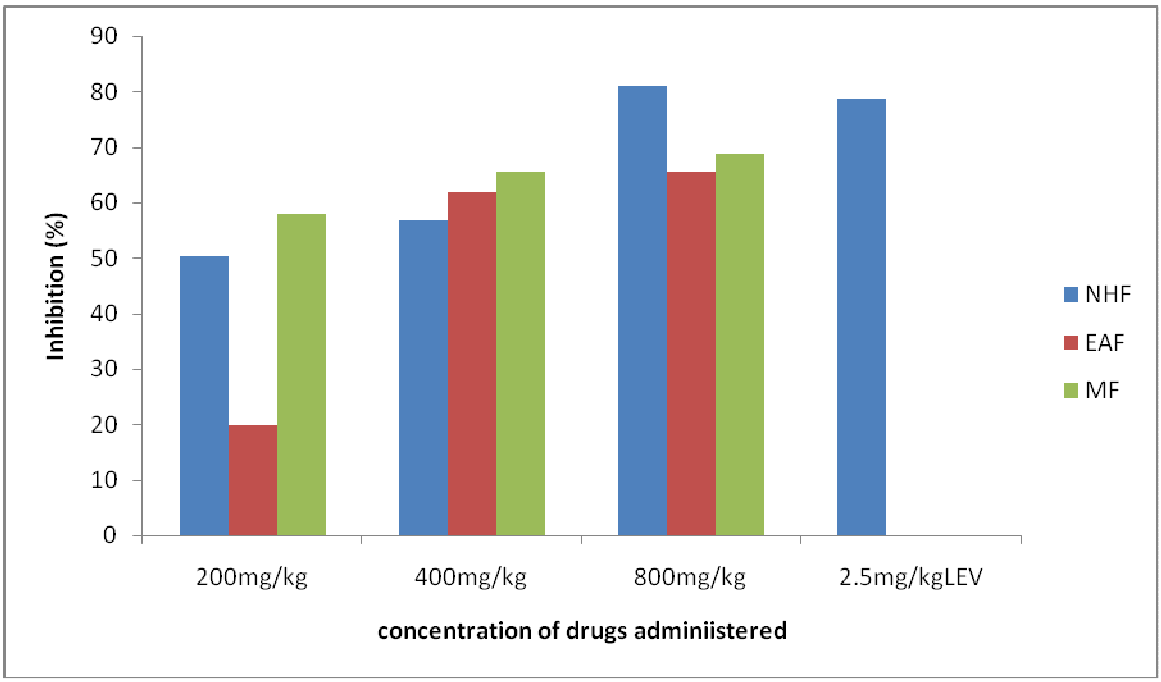


Figure A6: **Percentage Leucocyte Mobilization by ME**

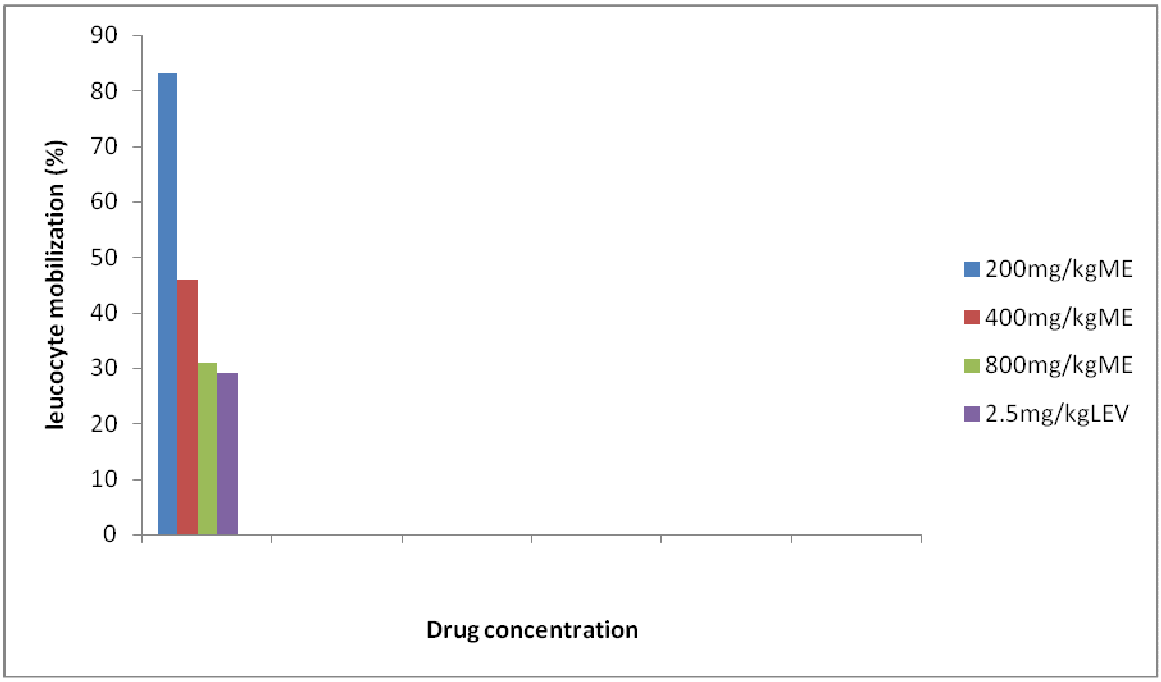
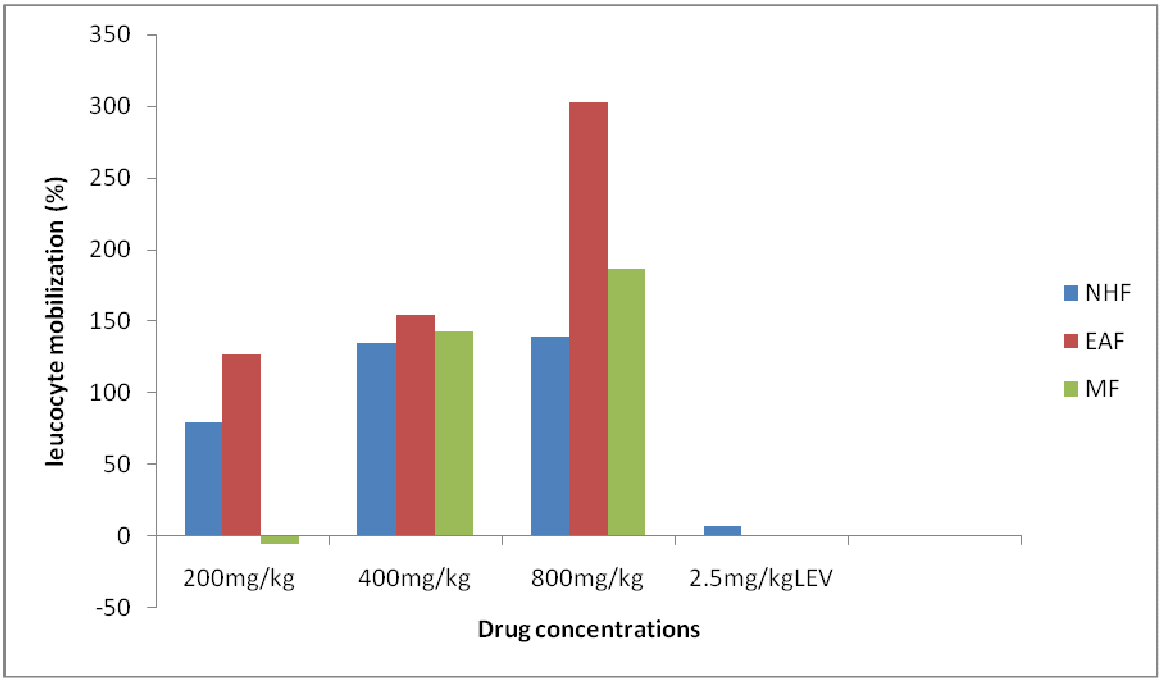


Figure A7: **Percentage Leucocyte Mobilization by the fractions**



REFERENCE

Ahsan,N., Hricik, D., Matas, A., Rose, S., Tomlanovich, S., Wilkinson, A., Ewell, M., Mclntosh,M., Stablein, D., Hodge, E. (1999). Prednisone withdrawal in kidney transplant recipients on cyclosporine and mycophenolate mophetil-a prospective randomized study. Steroid withdrawal study group. *Transplantation,* 68: 1865–1874.

Akah, P.A., Odo, C.I. (2010). Hepatoprotective effects of the solvent fractions of the stem of *Hoslundia opposita* Vahl. (Lamiaceae) against Carbon tetrachloride and paracetamol-induced liver damage in rats. *International Journal of Green Pharmacy,* 4(1): 54–58.

Akbar, A.N., Terry, L., Timms, A., Beverly, P.C., Janossy, G. (1988). Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells: *Journal of Immunology,* 140(7): 2171–2178.

Akolade, J.O., Muhammed, N.O., Usman, L.3., Owolarafe, T.A. Oloyede, O.B. (2011). Antidyslipidemic effect of leaf essential oil of *Hoslundia opposita* Vahl. In Allosan-induced diabetic rats. *International Journal of Tropical Medicine and Public Health,* 1(1): 1–29.

Alan, M.K., Terry, B.S., Jeffrey, A.B. (2001). Immunomodulators: immunosuppressive agents, tolerogens and immunostimulants. *Goodman and Gilman's The pharmacological basis of therapeutics*; 10th ed. United States: McGrawHill Companies.

Alberts, B., Alexander J., Julian L., Martin R., Keith R., Peter W. (2002). *Molecular Biology of the Cell.* (4th Edition). New York and London: Garland Science.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Water, P. (2002). *Molecular biology of the cell*. Garland science. New York. P. 1367.

Alder, M.N., Rogozin, I.B., Lyer, L.M/. Glazko, G.V., Cooper, M.D., Pancer, Z. (2005). Diversity and function of Adaptive Immune receptors in Jawless vertebrate.

Allen, B. (1999). Delayed type hypersensitivity: current theories with an historic perspective. *Dermatology Online Journal* 5(1): 7.

Al-Razi (2003). The Columbia Electronic Encyclopedia, 6th ed. Columbia University press.

Amirghofran, Z., Azadbakht, M., Karimi, M.H. (2000). Evaluation of the immunomodulatory effects of five herbal plants. *Journal of Ethnopharmacology,* 72(1-2): 167–172.

Anderson, N., Nester, R. (2007). *Microbiology: A human perspective*. (5th Ed.)

Andry, N. (1700). Germ theory calendar. 3rd ed. London. Rhodes and Bells printers.

Annan, K., Jackson, N., Dickson, R.A., Sam, G.H., Komlaga, G. (2011). Acaricidal effect of an isolate from *Hoslundia opposita* vahl against *Amblyomma variegatum*. *Pharmacognosy Research*, 3(3): 185-188.

Arbonnier, M. (2004). *Trees, shrubs and lianas of West African dry zones*. CIRAD; Paris.

Auphan, N., Didonato, J.A., Rosette, C., Helmberg, A., Karin, M. (1995). Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of 1 kappa B synthesis. *Science*, 270: 286–290.

Aw, D., Silva, A.B., Palmer, D.B. (2007). Immunosenescence: emerging challenges for an ageing population. *Immunology* 120(4): 435–446.

Ayensu, E.S., De Filipps, R.A. (1978). Endangered and threatened plants of the United States. Washington, D.C: Smithsonian Institution.

Babu T.D., Kuttan G., Padikkala J. (1995). Cytotoxic and anti-tumour properties of certain texa of Umbelliferae with special reference to *Centella asiatica*. *Journal of Ethnopharmacolology,* 48: 53–57.

Barakat, M.Z., Shahab, S.K., Darwin, N., Zahemy, E.L. (1993). Determination of ascorbic acid from plants. *Anal Biochemistry*, 52, 225–245.

Baron, S., Coppenhaver, D.H., Dianzani, F. (1992) Introduction to the interferon system. In: Baron, S., Dianzani, F., Stanton, G.J., Fleischmann, W.R., Jr. Coppenhaver, D.H., Hughes, T.K., Klimpel, G.R., Niesel, D.W., Tyring, S.K. (Eds.). *Interferon: Principles and Medicinal Applications* .Galveston, Texas, Pp. 1-15.

Benacerraf, R.A. (1978). Hypothesis to relate the specificity of T lymphocytes and the activity of I region Ir genes in macrophages and B lymphocytes. *Journal of Immunology,* 120: 1809–1812.

Bergstresser, P.R., Sullivan, S., Streilein, J.W., Tigelaar, R.E. (1985). Origin and function of Thy-1+ dendritic epidermal cells in mice. *Journal of Investigative Dermatology,* 85(1): 855–905.

Bertino, J.R. (1973). Chemical action and pharmacology of methotrexate, azathioprine and cyclophosphamide in man. *Arthritis & Rheumatology,* 16:79–83.

Biedermann, T.R., Maailhammer, A., Mai, C., Sander, A., Ogilivie, F., Brombacher, K., Maier, D., Rocken, M.L. (2001). Reversal of established delayed type hypersensitivity reactions following therapy with IL-4 or antigen specific Th2 cells. *Euopean Journal of Immunology*, 5: 1582–1591

Biswas , K., Chattopadhya, I., Banerjee, R. K., Banfyopadhyay U. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*) *Current Science*, 82(11): 1136–1345.

Black, C.A. (1999). Delayed type hypersensitivity: Current theories with an historic perspective. *Dermatology Online Journal,* 5(1): 7.

Bourdage, J.S., Hamlin, D.M. (1995). Comparative polyclonal antithymocyte globulin and antilymphocyte/antilymphoblast globulin anti-CD antigen analysis by flow cytometry. *Transplantation*, 59: 1194–1200.

Brown, P. (2001). Cinderella goes to the ball. *Nature,* 410: 1018-1020.

Bukowski, R.M. (1999). Immunotherapy in renal cell carcinoma. *Oncology (huntington*), 13: 801–810.

Burke, J.F. Jr., Pirsch, J.D., Ramos, E.L., Salomon, D.R., Stablein, D.M., Van Buren, D.H., West, J.C. (1994). Long-term efficacy and safety of cyclosporine in renal-transplant recipients. *New England Journal of Medicine*, 331: 358–363.

Cao, J., Park, I., Cooper, A., Sodroski, J. (1996). Molecular determinants of acute single-cell lysis by human immunodeficiency virus type 1. *Journal of Virology,* 70**:** 1340–1354.

Chabner, B.A., Ryan, D.P., Paz-AresL., Garcia-CarboneroR., Lalabnesi, P. (2001). Antineoplastic agents. *Goodman and Gilman's The pharmacological basis of therapeutics.* 10th ed. united states: McGrawhill, New York, p. 1395.

Chan, G.L., Canafax, D.M., Johnson, C.A. (1987). The therapeutic use of Azathioprine in renal transplantation. *Pharmacotherapy*, 7:165–177.

Chandra, R.K. (1997). Nutrition and the immune system: an induction. *The American Journal of Clinical Nutrition* 66(2): 4605–4635.

Chase, M.W., Landsteiner, K. (1940). Studies on the sensitization of animals with simple chemical compounds: VII. *Journal of Experimental Medicine,* 71(2): 237-245.

Chhabra, S.C., Uiso, F.C. (1994). Antibacterial activity of some Tanzanian plants used in traditional medicine. *Fitoterapia*, 62(6): pp 499–503

Codd, L.E. (1985). Lamiaceae. Flora of southern Africa. Botanical research Institute, Pretoria. Pp. 284.

Coico, R., Sunshine, G., Benjamin, E. (2003). *Immunology: A short Course*. Garland publishing New York. 5th ed. p. 48

Copeland, K.F., Heeney, J.L. (1996). T helper cell activation and human retroviral pathogenesis.

De M., Krishina De A., Baneriee A.B. (1999). Antimicrobial screening of some Indian species. *Phytother . Res.* pp 616–618.

Diasio, R.B., LoBuglio, A.F. (1996). Immunomodulators: Immunosuppressive agents and Immunostimulants. . In: Hardman J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W. Goodman, G.A. (Eds.). *Goodman and Gilman's The pharmacological basis of therapeutics*. 9th ed. McGraw-Hill, New York, pp 1291 – 1308.

Doberty, N.S. (1981). Selective effect of immunosuppressive agents against the delayed hypersensitivity response and humoral response to sheep red blood cells in mice. *Agents Actions,* 11:237–242.

Duff, G.W. (1985). Interleukin-1 and fever production. *Rheumatology,* 24(1)12–14.

Engels, F., Renirie, B.F., 'tHart, B.A., Labadie, R.P., Nikamp, F.P. (1992). Effect of apocynin, a drug isolated from roots of Picrorhiza kurroa on arachidonic acid metabolism. *FEBS Letters,* 305, 245–256.

Farnsworth N.R., Bunyapraphatsara N. (1992). *Thai medicinal plants: recommended for primary health care systems*. Pp 305.

Farquer, J.N. (1996). Plant sterols. Their Biological effects in humans. Handbook of lipids in human nutrition. BOCA. Paton FL CRC Press, pp: 101–105.

Faulds, D., Goa, K.L., Benfield, P. (1993). Cyclosporine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. *Drugs*, 45: 278–298.

Figueredo, A., Germond, C., Maroun, J., Brownman, G., Walker-Dilks, C., Wong, S. (1997). Adjuvant therapy for stage II colon cancer after complete resection. *Provincial Gastrointestinal Disease Site Group. Cancer Prevention Control*, 1: 379–392.

Firestein, G.S. (2007). The inflammatory response. In: Goldman L, Ausiello, D. eds. Cecil medicine. 23rd ed. Philadelphia, PG: saunders Elsevier; chap. 45.

Fuchimoto, Y., Yamada, K., Shimizu, A., Yasumoto, A., Sawada, T., Huang, C.H., Sachs, D.H. (1999). Relationship between chimerism and tolerance in a kidney transplantation model. *Journal of Immunology*, 162: 5704–5711.

Furr, S.R. (1998). Fundamentals of Immunobiology. In: *Pharmaceutical Microbiology*. Hugo, W.B., Russell, A.D. (Ed.), 6th Edn., Oxford: Blackwell Science Ltd., pp. 278-303.

Gaffney, K., Scot D.G. (1998). Azathioprine and cyclophosphamide in the treatment of rheumatoid arthritis. *British Journal of Rheumatology,* 37:824–836.

Gaffney, K., Scott, D.G. (1998). Azathioprine and cyclophosphamide in the treatment of rheumatoid arthritis. *British Journal of Rheumatology*, 37: 824–836.

Ganachari, M.S., Kumar, S., Bhat, K.G. (2004). Effect of ziziphus jujuba leaves extract on phagocytosis by human neutrophils. *Journal of Natural Redmedies,* 4: 47–51.

Gell, P.G.H., Coombs, R.R.A. (1963). *Clinical aspects of immunology*, 1st ed. Oxford, England: Blackwell.

Ghaffar, A. (2006). Hypersensitivity reactions. *Immunology.* Bookmark Printers. chap. 17

Gherardi, E. (from Answers.com, 2006). The concept of immunity, history and applications. Immunology course Medical school, University of Pavia. *The Columbia Electronic Encyclopedia*, 6th ed. Columbia University Press.

Glushakova, S., Grivel, J.C., Fitzgerald, W., Sylwester, A., Zimmerberg, J., Margolis, L.B. (1998). Evidence for the HIV-1 phenotype switch as a causal factor in acquired immunodeficiency. *Nature Medicine,* 4: 346–349.

Goronzy, J.J., Weyand, C.M. (2007). The innate and adaptive immune systems. In: Goldman L., Ausiello, D. eds. Cecil medicine. 23 ed. Philadelphia, Pa: Saunders Elsevier; chap. 42.

Gowans, J.L., McGregor, D.D., Cowen, D.M. (1962). Initiation of immune responses by small lynmphocytes. *Nature,* 196: 651–655.

Greenberg, S. (1957). A concise history of immunology: The role of smallpox in the development of vaccination. Chap. 5.

Grivel, J.C., Margolis**.** (1999). CCR5- and CXCR4-tropic HIV-1 are equally cytopathic for their T-cell targets in human lymphoid tissue. *Nature Medicine,* 5: 592–593.

Guermonprez, P., Valladeau, J., Zituogel, L., Theyr, C., Amigorena, S. (2002). Antigen presentation and T cells stimulation by dendritic cells. *Annual review of Immunology*, 20(1): 621–67.

Guo, Y., Lee, C., So, K.H., Gao, J., Yeung, W.S.B., Yao, Y., Lee, K. (2013). Human Leukocyte Antigen-Gs activates extracellular signal-regulated protein kinase signaling and stimulates trophoblast invasion. DOI: 10.1371/Journal. Pone.0076023.

Gupta, B., Pramanik, S., Tiwari, O.P., Thacker, N., Pande, M., Upmanyu, N. (2010). Immunomodulatory activity of Gymnema Sylvestre leaves. *The internet Journal of Pharmacology*, 8(2): 10.

Hale, D.A., Gottschalk, R., Umemura, A., Maki, T., Monaco, A.P. (2000). Establishment of stable multilineage hematopoietic chimerism and donor-specific tolerance without irradiation. *Transplantation*, 69: 1242–1251.

Hanabuchi, S., Ito, T., Park, W.R., Watanabe, N., Shaw, J.C., Roman, E., Arima, K., Wang, Y.H., Voo, K.S., Lao, W., Liu Y.J. (2010). Thymic stromal lymphopoietin-activated plasmacytoid dendritic cells induce the generation of FOXP3+ regulator T cells in human tumour. *Journal of Immunology,* 184161: 2999–3007.

Harborne, J.B.C (1984). *Phytochemical methods: a guide to modern technique of plant analysis*. (2nd Ed.). Chapman and Hall Ltd., London. Pp.282.

Hasket, R.F. (1985). Diagnostic categorization of psychiatric disturbance in Cushing's syndrome. *American Journal Psychiatry*, 142: 911-916.

Hayden, F.G. (2001). Antimicrobial agents continued, Antiviral agents (Non reetroviral) In: Hardman J.G., Limbird, L.E. (Eds.), *Goodman and Gilman's The pharmacological basis of therapeutics*. 10th ed. New York: McGraw-Hill, p 1346.

Haynes, B.F., Markert, M.L., Sempowski, G.D., Patel, D.D., Hale, L.P. (2000). The role of the thymus in immune reconstitution in aging, bone marrow transplantation and HIV-1 infection. *Annual Review of Immunology,* 18: 529–560.

Hedberg, I., Hedberg, O., Madati, P.J., Mshigeni, K.E., Mshiu, E.N., Samuelson, G. (1983). Inventory of plants used in traditional medicine in Tanzania. *Journal of Ethnopharmacol*ogy, 9(1): 105–127.

Herbein, G., Van Lint, C., Lovett, J.L., Verdin, E. (1998). Distinct mechanisms trigger apoptosis in human immunodeficiency virus type 1-infected and in uninfected bystander T lymphocytes. *Journal of Virology,* 72: 660–670.

Hillman, S.H. (2001). Hematopoietic agents, growth factors, minerals and vitamins. *Goodman and Gilman's The pharmacological basis of therapeutics.* 10th ed. McGrawhill, New York, p. 1494.

Hirose, R., Roberts, J.P., Quan, D., Osorio, R.W., Freise, C., Ascher, N.L., Stock, P.G. (2000). Experience with daclizumab in liver transplantation: renal transplant dosing without calcineurin inhibitors is insufficient to prevent acute rejection in liver transplantation. *Transplantation*, 69: 307–311.

Hoffmeister, R.T. (1983). Methotrexate therapy in rheumatoid arthritis: 15 years experience. *American Journal Medicine*, 75:69-73.

Holm, G.H., Zhang, C., Gorry, P.R., Peden, K., Schols, D., De Clercq, E., Gabuzda, D. (2004). Apoptosis of Bystander T cells induced by human immunodeficiency virus Type 1 with increased envelope/receptor affinity and co receptor binding site exposure. *Journal of Virology*, 78(9): 4541-4551

Holtmeier, W., Kabelitz, D. (2005). Gamma delta T cells link innate and adaptive immune responses. *Chemical immunology and allergy* 86: 151–183.

Hong, J.C., Kahan, B.D. (1999). Use of anti-CD25 monoclonal antibody in combination with rapamycinto eliminate cyclosporine treatment during the induction phase of immunosuppression. *Transplantation*, 68: 701–704.

Hong, J.C., Kahan, B.D. (2000). Immunosuppressive agents in organ transplantation: past, present and future. *Seminars in Nephrolology*, 20: 108–125.

Hunt, R., Mitzi, N. (2004). *Microbiology and Immunology online*. University of South Carolina.

Ingolfsdottir, K., Jureie, K., Fischer, B., Wagner, H. (1994). Immunomodulatory active polysaccharide from Cetraria islandica. *Planta Medica* 60, 527–531.

Inohara, I., McDonald, C., Nuñez, G. (2005). "NOD-LRR proteins: role in host-microbial interactions and inflammatory disease". *Annual Review of Biochemistry,* 74: 355–383.

Intayot Y., Kinouchi T., Kataoka K. (2002). Antimutagenicity of Murdannia loriformis in the Salmonella mutation assay and its inhibitory effects on azoxymethane-induced DNA methylation and aberrant crypt focus formation in male F344 rats. *Journal of investigative Medicine,* 49: 25–34.

Iwasaki, A., Medzhitov, R. (2010). Regulation of adaptive immunity by the innate immune system. *Science* 327: 291–295.

Iwu , M.M., Igboko, A.O. (1982). The flavonoids of Garcinia kola. *Journal of Natural Products*, 45: 650–651.

Iwu, M.M. (1993). *Handbook of African Medicinal plants*. Boca Raton, Florida: CRC Press. P. 192.

Jacobson, J.M., Greenspan, J.S., Spritzler, J., Ketter, N., Fahey, J.L., Vasquez, G.J., Whol, D.A. (1997). Thalidomide for the treatment of oral aphthous ulcers in patients with human immunodeficiency virus infection. National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group. *New England Journal of Medicine,* 336: 1487–1493.

Janeway C. A., (2005). *Immunobiology.* (6th ed.). Garland Science.

Janeway, C., Travers, P., Waalport, M., Shlomchik, M. (2001). *Immunobiology*: 5th ed. New York and London Garland Science. Chap. 1 & 2.

Jekle, A., Keppler, O.T., De Clercq, E., Schols, D., Weinstein, M., Goldsmith, M.A. (2003). In vivo evolution of human immunodeficiency virus type 1 toward increased pathogenicity through CXCR4-mediated killing of uninfected CD4 T cells. *Journal of Virology,* 77:5846–5854.

Jerner, E. (1955). The natural selection theory of antibody formation. *Proceedings of the Natural Academy of Sciences,* 41: 849–857.

Jiang, H., Chess L. (2004). An integrated viewof suppressor T cell subsets in immunoregulation. *Journal of clinical Investigation,* 114(9): 1198–1208.

Jide,O.A., Awe, S.O., Makinde, J.M. (1999). *Journal of Phytotherapy Research,* 13(5): 425–426.

Johnson, H.M., Bazer, F.W., Szente, B.E., jarpe, M.A. (1994). How interferon fight disease. *Scientific American*, 270: 68–75.

Joos, L., Tamm, M. (2005). Breakdown of pulmonary host defence in the immunocompromised host: cancer chemotherapy. *Proceedings of the American Thoracic society* 2(5): 445–448.

Kahan, B.D., Julian, B.A., Pescovitz, M.D., Vanrenterghem, Y., Neylan, J. (1999a). Sirolimus reduces the incidence of acute rejection episodes despite lower cyclosporine doses in Caucasian recipients of mismatched primary renal allografts: a phase II trial. Rapamune Study Group. *Transplantation*, 68: 1526–1532.

Kahan, B.D., Rajagopalan, P.R., Hall, M. (1999b). Reduction of the occurrence of acute cellular rejection among renal allograft recipients treated with basiliximab, a

chimeric anti-interleukin-2-receptor monoclonal antibody. United States Simulect Renal Study Group. *Transplantation*, 67: 276–284.

Kaiser, G.E. (2009). Interleukin-1 and fever. *Microbiology*, 11(5): 780–795.

Kapoor, R., Viijan, V., Singh, P. (2008). Bacillus Calmette Guerin in the management of superficial bladder cancer. *Indian Journal of Urology*, 24(1): 72-76.

Katja, H., Arnette, B., Elena, C., Simone, R., Ulrich, G., Werner, S., Friedrich, G., Ulf, S. (2002). Wegener's granulomatosus: antiproteinase 3 antibodies induce monocyte, cytokine and prostanoid release- role of autocrine cell activation. *Journal of Leukocyte Biology,* 71: 996–1004.

Kendall, M. (2007). Fine airborne urban particles (pm 2.5) sequester lung surfactant and amino acids from human lung lavage. *Journal of physiology,* 293(4): 1053–8.

Khazan, W., M'bika, J.P., Tomkiewicz, C. (2005). Expression of macrophage-selective markers in human and rodent adipocytes. *FEBS Lett*ers, 579(25): 5631–5634.

Khoury, S., Sayegh, M.H., Turka, L.A. (1999). Blocking costimulatory signals to induce transplantation tolerance and prevent autoimmune disease. *International Reviews of Immunology*, 18: 185–199.

Kiessling, R., Klier, E., Pross, H., Wigzell, H. (1975). Natural killller cells in the mouse II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cells. *European Journal of Immunology,* 5: 117–121.

Kimball, J.A., Pescovitz, M.D., Book, B.K., Norman, D.J. (1995). Reduced human IgG anti-ATGAM antibody formation in renal transplant recipients receiving mycophenolate mofetil. *Transplantation*, 60:1379–1383.

Kirk, A.D., Harlan, D.M., Armstrong, N.N., Davis, T.A., Dong, Y., Gray, G.S., Hong, X., Thomas, D., Fechner, J.H. Jr., Knechtle, S.J. (1997). CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proceedings of the Natural Academy of Sciences,* 94: 8789–8794.

Kofi, A., Nora, J., Rita, A.D., George, H., Sam, I., Gustav, K. (2011). Acaricidal effect of an isolate from *Hoslundia opposita* vahl against Amblyomma variegatum (Acari: Ixodidae). *Pharmacognosy Research,* 3(3): 185–188.

Kone, W.M., Koffi, A.G., Bomisso, E.L.,Tra Bi F.H. (2012). Ethnomedical study and Iron content of some medicinal herbs used in traditional medicine in Cote D'Ivoire for the treatment of anaemia. *African Journal of traditional, complementary and alternative medicine*, 9(1).

Kovarik, J.M., Kahan, B.D., Rajagopalan, P.R., Bennett, W., Mulloy, L.L., Gerbeau, C., Hall, M.L. (1999). Population pharmacokinetics and exposure-response relationships for basiliximab in kidney transplantation. *Transplantation*, 68: 1288–1294.

Kreis, H., Cisterne, J.M., Land, W., Wramner, L., Squifflet, J.P., Abramowicz, D., Campistol, J.M., Morales, J.M., Grinyo, J.M., Mourad, G., Berthoux, F.C., Brattstrom, C., Lebranchu, Y., Vialtel, P. (2000). Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. *Transplantation*, 69: 1252–1260.

Krensky A.M., Strom T.B., Bluestone, J.A. (2001). Immunomodulators: Immunosuppressive agents, Tolerogens, and Immunostimulants. In*, Goodman and Gilman's, The Pharmacological Basis of Therapeutics*, 10th ed. McGraw Hill, New York. pp. 1463–1484.

Krensky, A.M., Weiss, A., Crabtree, G., Davis, M.M., Parham, P. (1990). T-lympphocyte-antigen interactions in transplant rejection. *New England Journal of Medicine*, 322: 510–517.

Krishnaswamy, G., Ajitawi, O., Chi, D.S. (2006). The human mast cell: an overview. *Methods in molecular biology* 315: 13–34.

Krombach, F., Munzing, S., Allmeling, A.M., Gerlach, J.T., Behr, J., Dooger, M. (1997). Cell size of alveolar macrophages: an interspecies comparison. *Environmental Health perspectives,* 105(5): 1261–3.

Kulkarni, S.R., Karande, V.S. (1998). *Journal of Indian drugs*. 35(7). Pp 427–433.

Kumar, S.V., Kumar, S.P., Rupesh, D., Nitin, K. (2011). Immunomodulatory effects of some traditional medicinal plants. *Journal of Chemical and Pharmaceutical Research*, 3(1): 675-684.

Kumar, S.V., Kumar, S.P., Rupesh, D., Nitin, K. (2011). Immunomodulatory effects of some traditional medicinal plants. *Journal of Chemical and Pharmaceutical Research,* 3(1): 675-684.

Kuo, C.J., Chung, J., Fiorentino, D.F., Flanagan, W.M., Blenis, J., Crabtree, G.R. (1992). Rapamycin selectively inhibits interleukin -2 activation of p70S6 kinase. *Nature,* 358:70–73.

Kuo, C.J., Chung,J., Fiorentino, D.F., Flanagan, W.M., Blenis, J., Crabtree, G.R.(1992). Rapamycin selectively inhibits interleukin-2 activation of p70 S6 kinase. *Nature*, 358: 70–73.

Lane, N.E., Lukert, B. (1998). The science and therapy of glucocorticoids induced bone loss. *Endocrinology Metabolism Clinics of North America*, 27: 465-483.

Larsen, C.P., Alexander, D.Z., Hollenbaugh, D., Elwood, E.T., Ritghie, S.C., Aruffo, A., Hendrix, R., Pearson, T.C. (1996). CD40-gp39 interactions play a critical role during allograft rejection. Suppression of allograft rejection by blockade of the CD40-gp39 pathway. *Transplantation*, 61:4–9.

Lary, J.M., Daniel, K.L., Erickson, J.D., Roberts, H.E., Moore, C.A. (1999). The return of thalidomide: can birth defects be prevented? *Drug safety*. 21:161–169.

Lauralee, S. (2004). *Human physiology: From cells to systems*. 7th ed. London: West publishing company. Pp 450–461.

Levin, R.E., Kalidas, S., Gopinadhan, P., Pometto, A. (2006). *Food biotechnology,* 2nd ed. CRC press, USA. P 1028.

Lindquester , G.J. (2006). Introduction to the history of disease. Disease and Immunity. USA: Rhodes College. Chap. 1–3.

Lo Iacono, O., castro, A., Diago, M., Moreno, J.A., Fernandez-bermejo, M., Vega, P., Garcia-Buey, L., Garcia-monzon, C., Pedreira, J., Moreno-Otero, R. (2000). Interferon alfa-2b plus ribavirin for chronic hepatitis C patients who have not responded to interferon monotherapy. *Ailment*ary *Pharmacology & Therapeutics*, 14: 463–469.

Lo,T.N., Almeida, A.P., Beaven, M.A. (1982). Dextran and carrageenan evoke different inflammatory responses in rats with respect to composition of infiltrates and effect of indomethacin. *Journal of pharmacology and experimental therapeutics*, 221:261–267.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology,* 53: 275.

Lu, Y., Koga, Y., Tanaka, K., Sasaki, M., Kimura, G., Nomoto, K.(1994). Apoptosis induced in $CD4^+$ cells expressing gp160 of human immunodeficiency virus type 1. *Journal of Virology,* 68: 390–399.

Madigan, M., Martinko, J. (2005). *Biology of microorganisms*. 11th ed. USA: Prentice Hall. Chap. 7.

Male, D., Brostoff, J., Roth, D., Roitt, I. (2006). *Immunology*. 7th ed. pp 19–28.

Mansour, S.M. (2008). Invariant Natural Killer T cells in Immune Surveillance and Tumor Immunotherapy: Perspectives and Potentials. *Archives of Iranian Medicine*. 11(2): 186–195.

Mariat, C., Alamartine, E., Diab, N., de Filippis. J.P., Laurent, B., Berthoux, F.A. (1998). A randomized prospective study comparing low-dose OKT3 to low-dose ATG for the treatment of acute steroid –resistant rejection episodes in kidney transplant recipients. *Transplant International*, 11: 231–236.

Mayer, A.D., Dmitrewski, J., Squifflet, J.P., Besse, T., Grabensee, B., Klein, B., Eigler, F.W., Heemann, U., Pichlmayr, R., Behrend, M., Vanrenterghem, Y., Donck, J., Van Hooff, J., Christiaans, M., Morales, J.M., Andres,A., Johnson, R.W., Short, C., Buchholz, B., Rehmert, N., land, W., Scleibner, S., Forsythe, J.L., Talbot, D., Neumayer, H.-H., Hauser, I., Ericzon, B.-G., Brattstrom, C., Claesson, K., Muhlbacher, F., Pohanka, E. (1997). Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: a report of the European Tacrolimus Multicenter Renal Study Group. *Transplantation*, 64: 436–443.

McCune, J.M. (2001). The dynamics of $CD4^+$ T-cell depletion in HIV disease. *Nature*, 410**:**974–979.

Medzhitov, R., Janeway, C. (2000). Innate Immunity. Outstanding clear coverage of the mechanisms involved in innate immunity and its significance for the adaptive immune response *New England Journal of Medicine,* 343: 338-344.

Miller, J.F. (1993). Self-non self discrimination and tolerance in T and B lymphocytes. *Immunologic research* 12(2): 115–130.

Miller, J.F. (2002). The discovery of thymus derived lymphocytes. *Immunological Reviews* 185(1): 7–14.

Miller, M.T., Stromland, K. (1991). Teratogen update: thalidomide: a review, with a focus on ocular findings and new potential uses. *Teratology*, 60: 306–321.

Mitchell, R.S., Kumar, V., Abbas, A.K., Fausto, N. (2007). *Robbins basic pathology*. 8th ed. Philadeiphia: Saunders. Pp 431–436.

Moertel, C.G., Flemming, T.R., Macdonald, J.S., Haller, D.G., Laurie, J.A., Goodman, P.J., Ungerleider, J.S., Emerson, W.A., Tourney, D.C., Glick, J.H., Veeder, M.H., Mailliard, J.A. (1990). Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. *New England Journal of Medicine*, 322: 352–358.

Morales, A., Ottenhof, P., Emerson, L. (1981). Treatment of residual, non-infiltrating bladder cancer with bacillus Calmette-Gueri., *Journal of Urology,* 125: 649–651.

Morton, J.F. (1981). *Atlas of medicinal plants of middle America: Bahamas to Yucatan Sprigfield.* Illinois, USA, pp. 745–750.

Moshi, M.J., Otieno, D.F., Mbabazi, P.K., Weisheit, A. (2009). The ethnomedicine of the Haya people of Bugabo ward, Kagera region, North western, Tanzania. *Journal of ethnobiology and ethnomedicine*, 5: 24.

Murphy, B., Krensky, A.M. (1999). HLA-derived peptides as novel immunomodulatory therapeutics. *Journal of the American Society of Nephrology*, 10: 1346–1355.

Nat, V.D., Klerx, J.P., Dijk, V.H., Desilva, K.T. (1987). *Journal of Ethnopharmacology*, 19. Pp 125–131.

Natsumeda, Y., Carr, S.F. (1993). Human type 1 and 11 IMP dehydrogenases as drug targets. *Annals of the New York Academy of Science*, 696: 88–93.

Netea, M.G.,Van der G.C., Van der Meer J.W.M., Kullberg, B. (2004). The role of Toll-like receptors in host defense: bringing specificity to the innate immune response. *Journal of Leukocyte Biology*, 75: 749–755.

Ngadjui, B.T., Ayafor, J.F., Sondengam, B.L., Connolly, D.J., Rycroft, D., Tillequim, F. (1991). Phytochemistry 32(5). 1313-1315. Hoslundin, hoslundal, and hoslunddiol: three new flavonoids from the twigs of *Hoslundia opposita* (Lamiaceae). *Tetrahedron*, 47, 3555–3564.

Niedzwiecki, A., Rasnick, D., Mhlongo, S., Ratt, M.R.D. (2005). The leading cause of Immune deficiency disease worldwide. *Dr Ratt's cellular health series*.

Ogunleye, D.S., Ibitoye, S.F. (2003). Studies of antimicrobial activity and chemical constituents of Ximenia Americana. *Tropical Journal of Pharmaceutical Research*, 2(2): 239–241.

Ogura, M., Cordell, G.A., Farnsworth, N.R. (1977). Llodia 40: 157

Okwu D., Omodamiro, O.D. (2005). Effect of hexane extract and phytochemical content of Xylopia ethiopia and Ocimum aratissimum on the uterus of guinea pig. *Biological Research*, p 3.

Olajide, O.A., Oladiran, O.O., Awe, S.O., Makinde, J.M. (1998). Pharmacological evaluation of *Hoslundia opposita* extract in rodents. *Pharmacotherapy research*, 12(5). Pp 364–366.

Oldham, R. K. (1983). Natural killer cells: Artifact to reality. *Cancer and Metastasis Reviews*, 2(4): 323–336.

Opelz, G., Terasaki, P.I. (1978). Improvement of kidney-graft survival with increased numbers of blood transfusions. *New England Journal of Medicine,* Med. 229:799-803.

Opelz, G., Vanrenterghem, Y., Kirste, G., Gray, D.W., Horsburgh, T., Lachance, J.G., Largiader, F., Lange, H., Vujaklija-Stipanovic, K., Alvarez-Grande, J., Schott, W., Hoyer, J., Schnuelle, P., Descoeudres, C., Ruder, H., Wujciak, T., Schwarz, V. (1997). Prospective evaluation of pretransplant blood transfusions in cadaver kidney recipients. *Transplantation*, 63: 964–967.

Ortho Multicenter Transplant Group. (1985). A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. *New England Journal of Medicine,* 313: 337–342.

Parham, P. (2005). *The Immune system*. Garland Science, New York.

Patard, J.J., saint, F., Velotti, F., Abbou, C.C., Chopin, D.K. (1998). Immune response following intracesical bacillus Calmette- Guerin instillations in superficial bladder cancer: a review, *Urological Research*, 26:155–159.

Paterson, D.L., Patel, A. (1998). Bacillus Calmete-Guerin (BCG) immunotherapy for bladder cancer: review of complications and their treatment. *Australian and New Zealand Journal of Surgery*, 68: 340–344.

Patwardhan, B., Kalbag, D., Patki P.S., Nagsampadi, B.A. (1990). Search of Immunomodulatory agents: a review. *India drugs*, 28(2): 348–358.

Pickering, H., Roe, E. (2009). *Wild flowers of the Victoria falls Area*. Helen Pickering, London. P. 67.

Pilat, N.I., Hock, K., Wekerle, T. (2012). Mixed cell chimerism through donor bone marrow transplantation: a tolerogenic cell therapy for application in organ transplantation. *Current Opinion in Organ Transplantation*, 17(12): 63-70.

Pooley, E. (1998). *A field guide to wild flowers kwaZulu-Natal and eastern Region*. Natal Flora Publication Trust, Durban.

Priyanka,S., Mansi, V., Jha, K.K., Maanju, P. (2012). *Journal of Advanced Scientific Research*, 3(1): 7–12.

Punt, C.J. (1998). The use of interferon- alpha in the treatment of cutaneous melanoma: a review. *Melanoma Research*, 8:95–104.

Punturee, K., Kasinrerk, W., Wild, C.P., Vinitketkumnen, U. (2005). Immunomodulatory effects of Thai medicinal plants. *Chiang Mai Medbull,* 44(1): 1-12.

Rang, H.P., Dale, M.M., Ritter, J.M., Flower, R.T. (2007). Local Hormones, Inflammation and Immune reactions. In: *Pharmacology*. 6th ed. Elsevier, London. Pp 203–247.

Ransohoff, R.M. (1998). Cellular responses to interferons and other cytokines: the JAK-STAT paradigm. *New England Journal of Medicine*, 338: 616–618.

Ratt, I.M; Brostoff, J.; Male, D.K. (2001). *Immunology*, 6th edition. Elsevir science: Philadelphia, pp. 1–440

Regan, T.V. (2003). *The Gell-Coombs classification of hypersensitivity rections: a re-interpretation*.8th ed. saunders.

Reuter H.D., (1995). Allium sativum and Allium ursinum: part 2. Pharmacology and medicinal application. *Phytomedicine,* 2: 73–91.

Revenis, M.E., Kaliner, M.A. (1992). Lactoferrin and lysozyme deficiency in airway secretions: association with the development of bronchopulmonary dysplasia. *Journal of Pediatrics,* 121 (2): 262-270.

Ribeiro, R.A., Flores, C.A., Cunha F.Q., Ferreira, S.H. (1991). IL-8 causes *in vivo* neutrophil migration by a cell dependent mechanism. *Immunology*, 73: 472–477.

Risa, J., Risa, A., Adsersen, A., Gauguin, B., Stafford, G.I., Van, S.J., Jager, A.K. (2004). Screening of plants ued in southern Africa for epilepsy and convulsions in GABA A-benzodiazepine receptor assay. *Journal of Ethnopharmacology*, 93, 177–182.

Roitt, I., Brostoff, J.., Male, D. (2001). *Immunology* (6th ed.) pp 480.

Rostaing, L., Chabannier, M.H., Modesto, A., Rouzaud, A., Cisterne, J.M., Tkaczuk, J., Durand, D. (1999). Predicting factors of long - term results of OKT3 therapy for steroid resistant acute rejection following cadaveric renal transplantation. *American Journal of Nephrology*, 19: 634–640.

Rothenberg, M., Hogan, S. (2006). The Eosinophils. *Annual Review of Immunology*, 24(1): 147-174.

Sabatino, D.E., Nichols, T.C., Merricks, E., Bellinger, D.A., Herzog, R.W., Monahan P.E. (2012). Annual models of hemophilia; progress in molecular biology and Translational Science 105: 151–209.

Sampaio, E.P., Kaplan, G., Miranda, A., Nery, J.A., Miguel, C.P., Viana, S.M., Sarno, E.N. (1993). The influence of thalidomide on the clinical and immunologic manifestation of erythema noodosum leprosum. *Journal of Infectious Diseases*, 168: 408–414.

Sanchez, L., Calvo, M., Brock, J.H. (1992). *Biological role of lactoferrin Archives of Disease in Childhood*, 67(5): 657-661.

Sasaki, T., Shibata, S., Hirabayashi, Y., Sekiguchi, Y., Yoshinaga, K. (1989). Accessory cell activity of monocytes in anti- DNA antibody production in systemic lupus erythematosus. *Clinical & Experimental Immunology*, 77(1): 37–42.

Schreiber, S.L., Crabtree, G.R (1992). The mechanism of action of cyclosporine A and FK506. *Immunology Today,* 13: 136–142.

Schwarz B.A., Bhandoola, A. (2006). Trafficking from the bone marrow to the thymus: a prerequisite for thymopoiesis. *Immunological Reviews,* 209: 47–57.

Sharma, M.L., Rao, C.S., Duda, P.L. (1994). Immunostimulatory activity of Picrorhiza Kurnoa leaf extract. *Journal of Ethnophamacology,* 41: 85–192.

Silverstein, A.M. (1989). *History of immunology*. London: Academic Press. Chap. 1.

Singh, M., O'Hagan, D. (1999). "Advances in vaccine adjuvants". *Nature Biotechnology,* 17 (11): 1075–81.

Sinkovics, J.G., and Horvath, H.C. (2000). Vaccination against human cancers (review). *International Journal of Oncology*, 29: 81–96.

Smithells, R.W., Newman, C.G. (1992). Recognition of thalidomide defects. *Journal of Medical Genetics*, 29: 716–723.

Sofowora L.A. (1993). *Medicinal plants & traditional medicine in Africa*. Spectrum Books ltd, Ibadan, pp 55–71.

Sohni, Y.R., Bhatt, R. M. (1996). Activity of a crude extract formulation in experimental hepatic amoebiasis and in Immunomodulation studies. *Journal of Ethnopharmacology*, 54: 119–124.

Spitzer, T.R., Delmonico, F., Tolkoff-Rubin, N., McAfee, S., Sackstein, R., Saidman, S., Colby, C., Sykes, M., Sachs, D.H., Cosimi, A.B. (1999). Combined histocompatibility leukocyte antigen-matched donor bone marrow and renal transplantation for multiple myeloma with end stage renal disease: the induction of allograft tolerance through mixed lymphohematopoietic chimerism. *Transplantation*, 68: 480–484.

Sproul, T.W., Cheng, P.C., Oykstra, M.L., Pierce, S.K. (2000). A role for MHC class II antigen processing in B cell development. *International reviews of Immunology,* 19(2-3): 139–155.

Starzl, T.E., Demetris, A.J., Murase, N., Trucco, M., Thomson, A.W., Rao, A.S., Fung, J.J. (1997). Chimerism after organ transplantation. *Current Opinion in Nephrology and Hypertension*, 6: 292–298.

Stevenson, J.R. (2011). *Cells and organs of the immune system*. London: Rutledge. Chap. 2.

Stvrtinova, V., Jan, J., Ivan, H. (1995). *Inflammation and Fever from Pathophysiology: Principles of Disease.* Computing centre, Slovak Academy of science: Academic electronic press.

Suwannasaen, D., Romphruk, A., Leelayuwat, C., Leitmemongkolchai, G. (2010). Bystander T cells in human Immune responses to dengue antigens. *BioMed Central Journals of Immunol*, 20 (11): 47.

Takahashi, K., Kawaguchi, S., Nishimura, K.I., Kubota, K., Tanabe, Y., Takani, M. (1974). *Chemistry pharmaceutical bull*. 22: 650

Takiishi, T., Korf, H., Van Belle, T.L., Robert, S., Gysemans, Conny., Mathieu, C. (2012). Reversal of autoimmune diabetes by restoration of antigen-specific tolerance using genetically modified lactococcus lactis in mice. *Journal of clinical Investigation,* 122(5): 1717-1725.

Tilg, H., Kaser, A. (1999). Interferons and their role in inflammation. *Current Pharmaceutical Design*, 5: 771–785.

Tompkins, W.A. (1999). Immunomodulation and therapeutic effects of the oral use of interferon-alpha: mechanism of action. *Journal of Interferon & Cytokine Research,* 19: 817–828.

Trease, E.G., Evans, W.C. (1993). *Pharmacognosy.* Balliere Tindale, London, pp 278 - 539.

Undem, B.J., Lichtenstein, L.M. (2001). Drugs used in the treatment of asthma. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. (Joel F. Hardman and Lee E. Limbird eds) 10th ed. McGraw Hill, New York. Pp 734–735.

Vincenti, F. (2006). Costimulatory blockade-What will the future bring. *Transplantation*, 22(5): 1293-1296.

Vinitketkumnuen, U., Charoenkunathum W., Kongtawelert, P., Lertprasertsuke, N., Matsushima, T. (1996). Antimutagenicity and DT- diaphorase inducer activity of Thai medicinal plant, *Murdannia loriformis*. *Journal of Herbs, Species and Medicinal plants*, 4: 45–52.

Vinitketkumnuen, U., Puatanachokchai R., Kongtawelert, P., Lertprasertsuke, N., Matsushima, T. (1994). Antimutagenicity of lemon grass (*Cymbopogon citrates* Stapf) to various known mutagens in Salmonella mutation assay. *Mutation Research,* 341: 71–75.

Vinothapooshan, G., Sundar K. (2011). *African journal of Pharmacology,* 5(3), pp 306–310.

Vivier, E., Raulet, D.H., Moretta,A., Caligiuri, M.A., Zitvogel, L., Lanier, L.L., Yokoyama, W.M., Ugolini, S. (2011). Innate or adaptive imunity; the example of Natural killer cells. *Science,* 331:44–49.

Wang, J.Z., Mao, X.J., Ito, H., Shimura, K. (1991). Immunomodulatory activity of polysaccharide from Acanthopanex obovatus roots. *Planta medica,* 57: 335–336.

Warwick, R., Williams, P.L. (1973). *Angiology*. 5th ed. Longman, London. pp. 588–785.

Watanabe, N., Wang, Y.H., Lee, H.K., Ito, T., Wang, Y.H., Cao, W., Lui, Y. (2005) Hassal's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. *Nature,* 436:1181–1185.

Watt, J.M., Breyer-Brandwijk, M.G. (1962). *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. 2nd ed. Livingstone, London.

Weinstein, G.D. (1977). Methotrexate. *Annals of Internal Medicine*, 86: 199-204.

Wheater, P.R., Stevens, A. (2002). *Wheater's Basic Histopathology: a colour actions and text*. Edinburgh: Churchill livingstone.

Whittington, R., Faulds, D. (1993). Interleukin-2. A review of its pharmacological properties and therapeutic use in patients with cancer. *Drugs*, 46:

Willinger, T., Freeman, T., Hasegawa, H., McMichael, A.J., Callan, M.F. (2005). Molecular signatures distinguish human central memory from effector memory CD8T cell subsets. *Journal of Immunology*, 175 (9): 5895-5903.

Winkelhake, J.L., Gauny, S.S. (1990). Human recombinant interleukin-2 as an experimental therapeutic. *Pharmacological Reviews*, 42:1–28.

Woodle, E.S., Xu, D., Zivin, R.A., Auger, J., Charette, J., O'Laughlin, R., Peace, D., Jollife, L.K., Haverty, T., Bluestone, J.A., Thistlewaite, J.R. Jr. (1999). Phase I trial of humanized, Fc receptor non-binding OKT3 antibody, huOKT3gamma 1 (Ala-Ala) in the treatment of acute renal allograft rejection. *Transplantation*, 68: 608–616.

Worboys, M. (2008). *Spreading germs: Disease theories and medical practice in Britain, 1865-1900*. Cambridge: Cambridge University press.

Yang C.S., Wang Z.Y., Hong J.Y. (1994). Inhibition of tumorigenesis by chemical from garlic and teas. In: Jacobs MM (Eds.) *Diet and cancer: marker, prevention and treatment*. Plenum press, New York. Pp. 113–122.

Young, B., Lowe, J.S., Steven, A., Health, J.W. (2006). *Wheater's functional Histology* (5^{th} ed.)

Zen, K., Parkos, C.A. (2003). Leukocyte epithelial interactions. *Current Opinion in Cell Biology*, 15(5): 557–564.

Zoorob, R.J., Cender, D. (1998). A different look at corticosteroids. *American Family Physician*, 58: 443–450.

APPENDIX

Figure A1: Percentage Inhibition of DTH by ME

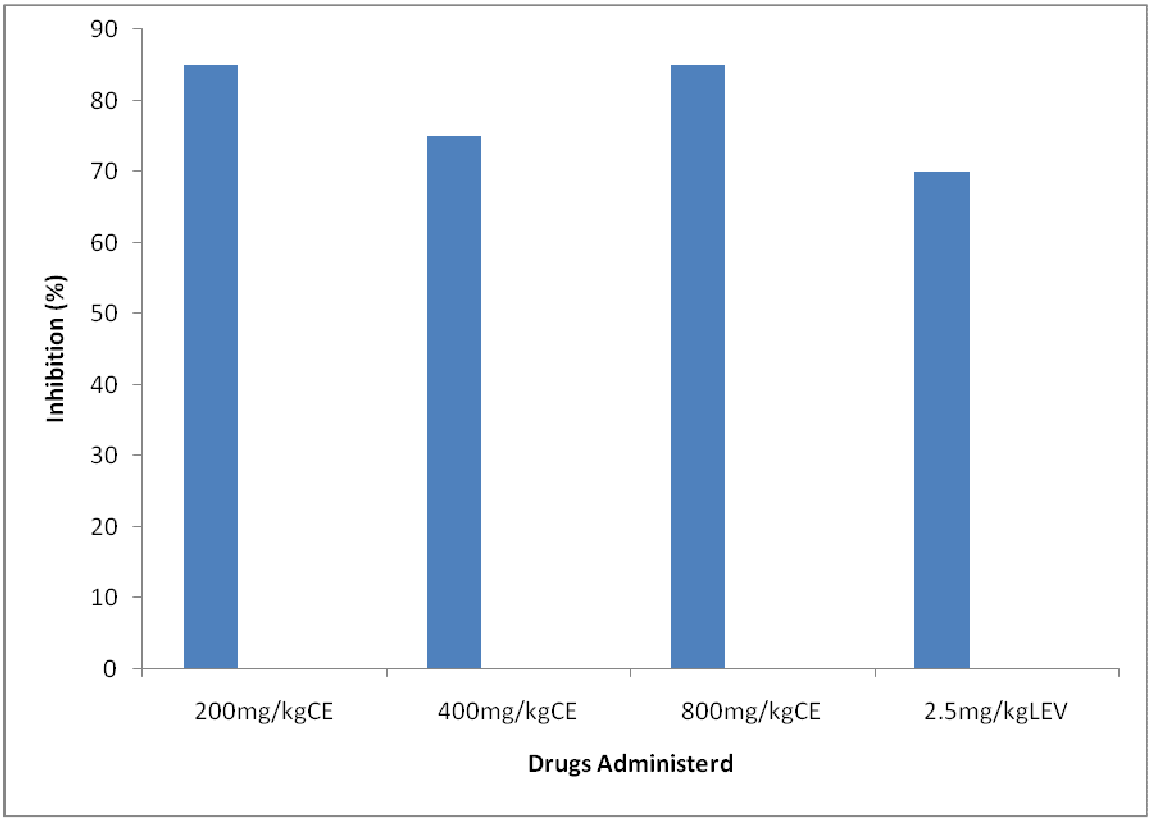


Figure A2: Percentage inhibition of DTH by NHF

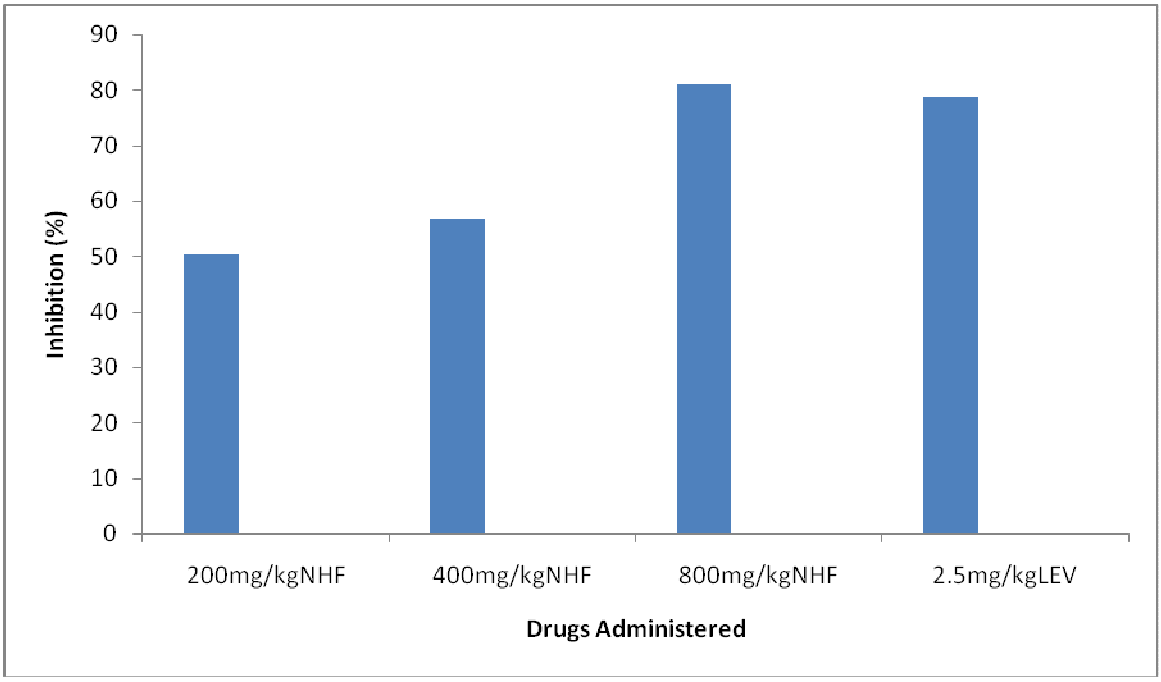


Figure A3: Percentage Inhibition of Delayed Type Hypersensitivity by EF

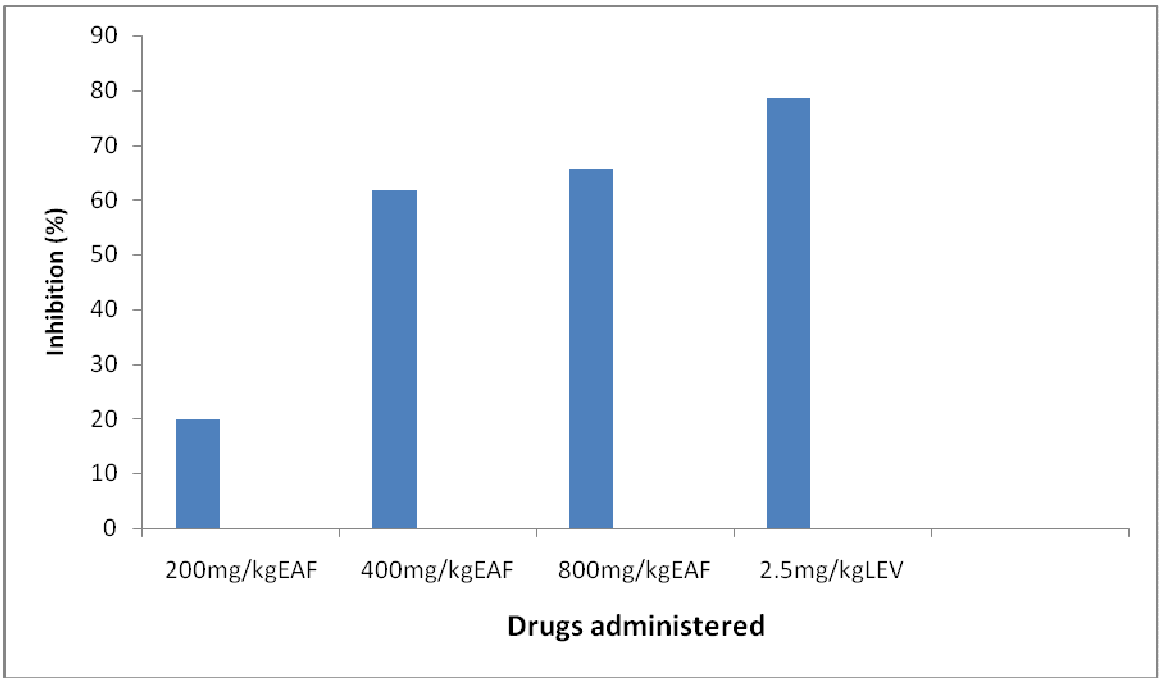


Figure A4: Percentage Inhibition of Delayed Type Hypersensitivity by MF

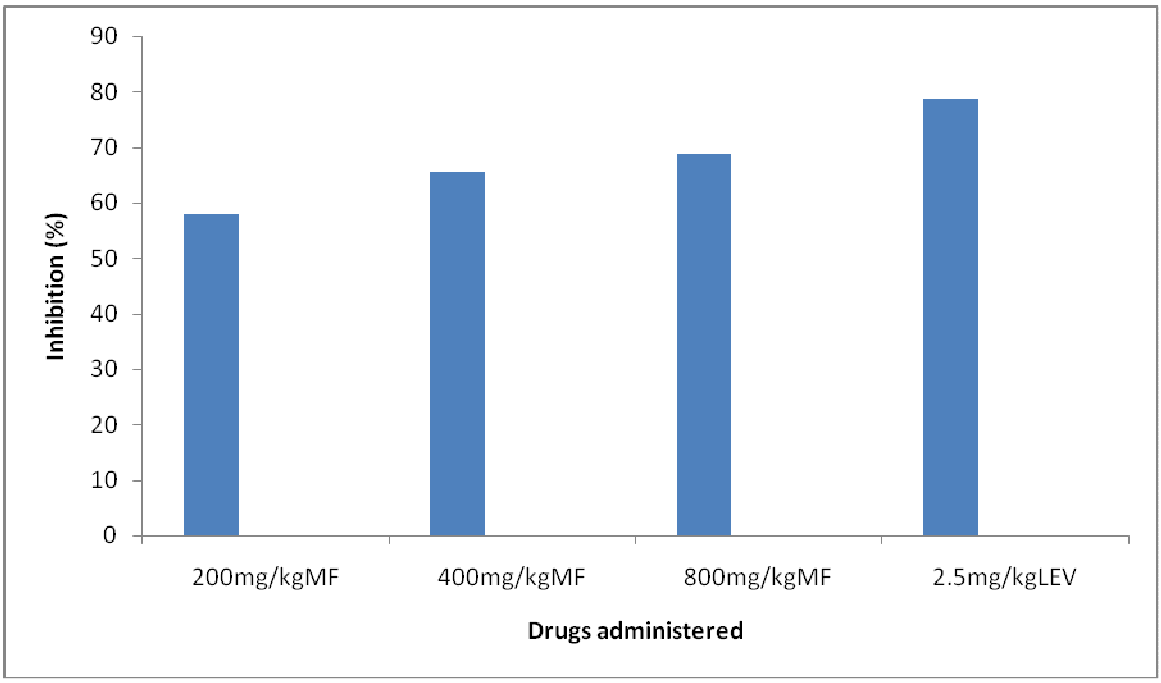


Figure A5: Percentage Inhibition of Delayed Type Hypersensitivity by the fractions

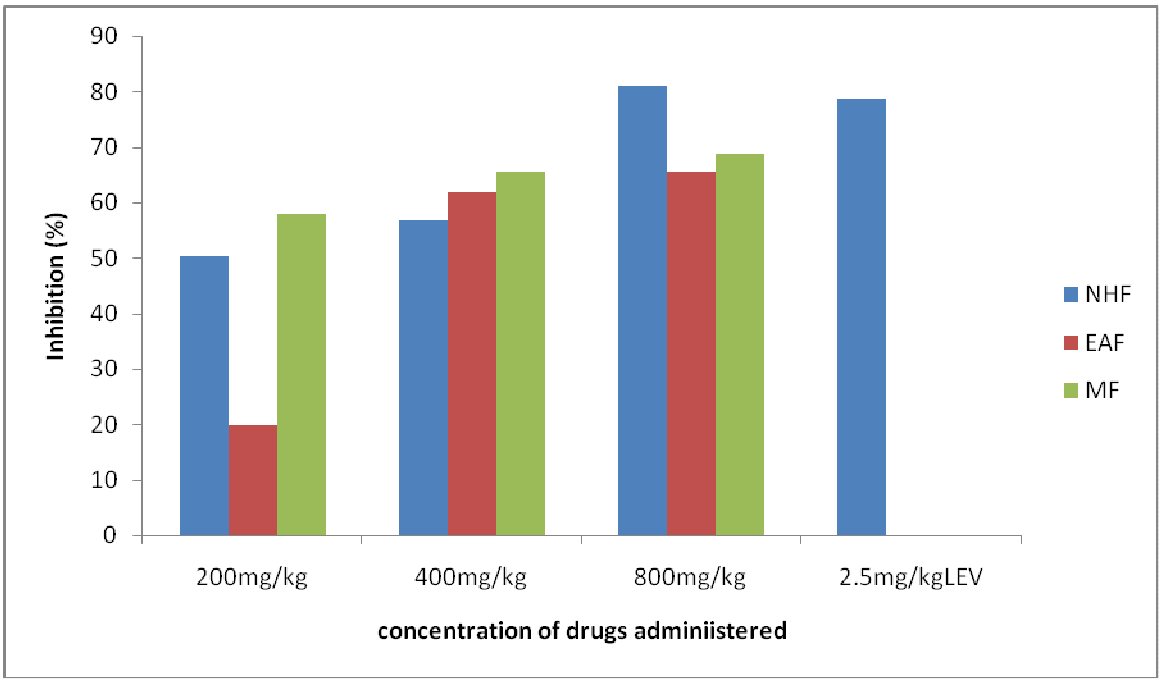


Figure A6: **Percentage Leucocyte Mobilization by ME**

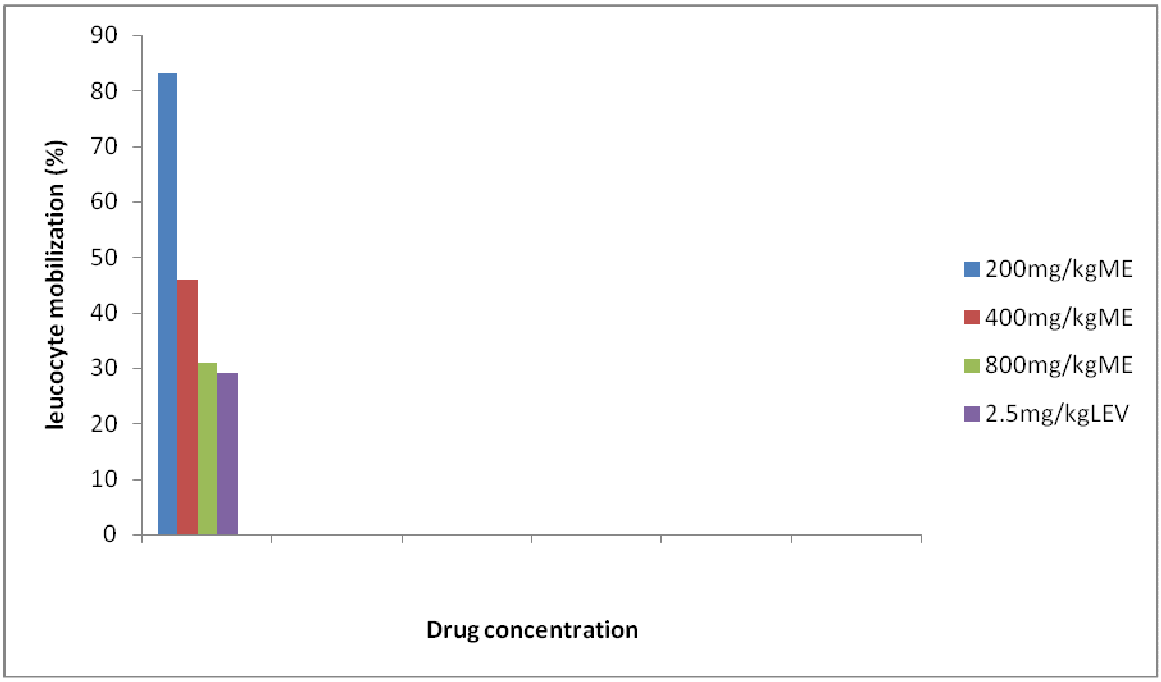


Figure A7: **Percentage Leucocyte Mobilization by the fractions**