IN VIVO ANTI-PLASMODIAL ACTIVITIES OF THE AQUEOUS AND METHANOLIC LEAF EXTRACTS OF *JATROPHA CURCAS* L. IN MICE INFECTED WITH *PLASMODIUM BERGHEI*

BY

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AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

APRIL, 2021

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DEPARTMENT OF ZOOLOGY,
FACULTY OF LIFE SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

APRIL, 2021

DECLARATION

I declare that the work in this thesis entitled "I***n vivo*anti-plasmodial activities of the aqueous and methanolic leaf extract of *Jatropha curcas* L. in mice infected with *Plasmodium berghei***"**has been carried out by me in the Department of Zoology, under the supervision of ProfessorI.S. Ndams and Prof. A.J. Natala. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

AKANDE Olalekan Ahmed _____ _____

Signature Date

CERTIFICATION

This thesis titled "***In vivo*** **anti-plasmodial activities of the aqueous and methanolic leaf extracts of *Jatropha curcas* L. in mice infected with*Plasmodium berghei*"** by Ahmed Olalekan AKANDE meets the regulations governing the award of the degree of Master of Science (M.Sc.) of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to all those who belief in me especially my mother (Orisa b'iya kosi) and my ever-supportive loving wife.

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ABSTRACT

In vivo anti-plasmodial activities of aqueous and methanolic fractions of the leaf extract of *Jatropha curcas* on *Plasmodium berghei* (NK-65)infected mice were investigated. The plant extracts were prepared by cold maceration using aqueous and methanol as a solvent. The crude methanol extract was partitioned using N-hexane, Ethyl acetate and N-butanol as a solvent. Phytochemical studies were carried out using preliminary phytochemical tests and Gas Chromatograph Mass Spectroscope to identify and quantify the active compound present in the plants extract. The antiplasmodial activity of the aqueous and methanolic leave extracts and its fraction was evaluated using two models, Peter four (4) days suppressive and curative tests.One-way ANOVA was used to determine the difference in activities of the extracts against *Plasmodium berghei*and haematological parameters. Qualitative phytochemical analysis showed the presence of saponin, flavonoid, alkaloids, tannin, triterpene, carbohydrates, steroids, glycosides and cardiac glycosides in aqueous extract, methanol extract, ethyl acetate fraction and butanol fraction while hexane fraction shows the presence of steroids, glycoside and cardiac glycosides. The Gas chromatography mass spectroscopy revealed the present of seventeen bioactive compounds of which Phytol (31.64%), Octadecanoic acid (18.30%) and 9,12,15-Octadecatrienoic acid (11.20%) recorded the highest percentage while Benzene, [1-(2,4-cyclopentadien-1- ylidene)ethyl] (0.53%) and Ethyl 9-decenoate (1.25%) were the least. The leaf extract caused no lethality in mice at oral LD_{50}value of $\leq$5000 mg/kg body weight. At the end of the four (4) days suppressive test, the effect of the aqueous and methanol extracts and its fractions of *Jatropha curcas* leaf on parasitaemia suppressive activity were dose-dependent for various extract treated group and crude methanol extract having the highest suppressive activity (67.15%) at 750mg/kg. In the curative test the Methanol extract and butanol fraction produced significant ($P<0.05$) reduction in the level of parasitaemia respectively. Aqueous and methanolic fractions of leaf extract *Jatropha curcas* significantly ($P<0.05$) protected the reduction of Red blood cell, Packed cell volume and White blood cell concentration in extract treated groups compared to the negative control group. The study haveshown that*Jatropha curcas* leaf has antimalarial activity and the extracts is safe for oral use. The study recommends that further research on aqueous and methanol extract and fractions of *Jatropha curcas* leaf could be carried out in order to isolate, identify and characterize the active compound from this plant.

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LIST OF ABBREVIATIONS

ABU: Ahmadu Bello University

ABUCAUC: Ahmadu Bello University Committee on Animal Use and Care

ANOVA: Analysis of Variance

APC: Average Parasitaemia in Control

APT: Average Parasitaemia in Treatment

CDC: Center for Diseases Control

CM: Cerebral Malaria

CQ: Choloroquine

DHA: Dihydroartemisinin

ELISA: Enzyme Linked Immunosorbent Assay

GC-MS: Gas Chromatograph Mass Spectroscopy

H & E: Haematoxylin and Eosin

IFA: Immunofluorescence Antibody

LD: Lethal Dose

NARICT: National Research Institute for Chemical Technology

NIMR: National Institute for Medical Research

PCV: Packed Cell Volume

RBC: Red Blood Cell

RDT: Rapid Diagnostic Test

SA: Severe Anaemia

WBC: White Blood Cell

WHO: Who Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Malaria is a debilitating disease that is transmitted to humans by the bite of female *Anopheles* mosquito infected by *Plasmodium* species(Mehlhorn, 2014). The mosquito bite introduces infective stage (sporozoite) of *Plasmodium* from the mosquito's saliva into the human blood (WHO, 2014). The parasites travel to the liver where they mature and reproduce. Five species of *Plasmodium* (*P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi*)can infect and be spread by humans with most death caused by *P. falciparum* (WHO, 2014). World Health Organization (2013) reported that about 3.4 billion people live in areas at risk of malaria transmission in 106 countries and territories. It was also reported that malaria caused 198 million clinical episodes and 500,000 deaths (Harvey and Fineberg, 2014).

Rollback malaria programme reported that malaria remained one of the major public problems in Sub-Sahara Africa (Tripathi, 2013). Moreover, the people at risk of this infectious disease are children under the age of five years and pregnant women (Uraku *et al.,* 2015). According to Ugwu *et al.* (2013), over 729 per 100,000 children of less than five years of age in Nigeria die annually due to malaria infection; the disease is prevalent in tropical and sub-tropical regions and is a major obstacle to economic advancement of the region's leading to poverty and malnutrition. In pregnant women, malaria often results in anemia, spontaneous abortion, pre-term babies with low-birth-weight and neonatal deaths among others. In 1955, the World Health Organization (WHO) began a program to eliminate malaria worldwide. Initially there was great success as WHO employed insecticides such as DDT against the mosquito vectors in some parts of the World. Unfortunately, strains of *Anopheles* mosquitoes began a rapid resurgence due to

resistance in the mosquitoes against DDT (WHO, 2005).Malaria infection has always been linked to change in climate, poverty, malnutrition, continuous resistance to the most available drugs and insecticides (Muller and Garenne, 1999). Susceptibility to malaria infection is sex independent as it is observed that both male and female have equal chance of acquiring the infection. However, some evidences suggests that in some countries, men have increased exposure because they spend more time sitting outside in the evenings during peak mosquito biting times (Vlassoff and Bonilla, 1994) and that some male-dominated types of work lead to increased exposure. For example, agricultural work extending to the evenings or sleeping away from settlements may raise risk, especially in forests, which can make men more vulnerable than women. (Erhart *et al.,* 2004; Incardona *et al.,* 2007).

Disease pathology varies with the intensity of infection and host immune response (Quellas,2011). The early manifestation of the disease, following 10-15 days of infection includes chills, headache, nausea and vomiting (Garcia and Lawas, 2008). As the disease progresses, some patients may develop the classic malaria paroxysm which may lead to death. The malaria paroxysm comprises three successive stages. The first is a 15 to 60-minute cold stage characterized by shivering and a feeling of cold. Next comes the 2 to 6-hour hot stage, in which there is fever, sometimes reaching 41°C, flushed, dry skin, and often headache, nausea, and vomiting, finally there is the 2 to 4 hour sweating stage during which the fever drops rapidly and the patient sweats. In a non-immune individual, *P. falciparum* infections are usually characterized by severe complications such as cerebral malaria (CM) and severe anaemia (SA) (Miller *et al*., 1994; Breman and Campell, 1998), other complications such as hypoglycaemia, renal failure and non-cardiogenic pulmonary oedema can occur singly or in any combination

(Miller *et al*., 1994; Sahu *et al*., 2010).Despite the substantial progress made in the treatment of parasitic diseases, malaria remains a significant therapeutic challenge especially because of the wide spread resistance of malaria parasites to currently available anti-malarial agents, the resistance of the mosquito vectors to currently available insecticides, the limited success in the development of malarial vaccines and the debilitating adverse reactions of conventional anti-malarial drugs. These have stimulated the search for new pharmacologically active agents that can overcome these barriers (Pretorius *et al*., 2013).

There is a long-standing tradition for the use of phytomedicines for the treatment of malaria. The plant kingdom remains a major target in the search of lead compounds and new drugs to treat this debilitating parasitic disease. Quinine isolated from *Cinchona* and quinghaosu from *Artemisiaannua* L. for instance illustrates the potential value of herbal medicines for development of antimalarial drugs. A major advance in the search for effective treatment for drug-resistant malaria came with the discovery of artemisinin and its derivatives. Severe malaria is treated with intravenous or intramuscularquinine or, since the mid-2000s, the artemisinin derivative artesunate, which is superior to quinine in both children and adults and is given in combination with a second anti-malarial such as mefloquine (Pretorius *et al.*, 2013). Resistance has been developed to several antimalarial drugs; for example, chloroquine-resistant *P. falciparum* has spread to most malarial endemic areas, and emerging resistance to artemisinin has become a problem in some parts of Southeast Asia. Artemisinin drugs, which originated from the Chinese herb qinghao (*Artemisia annua*), belong to a unique class of compounds, the sesquiterpene lactone endoperoxides. The parent compound of this class is artemisinin (quinghaosu), whereas dihydroartemisinin (DHA), artesunate, artemether, and β-artemether are

the most common derivatives of artemisinin. DHA is the main bioactive metabolite of all artemisinin derivatives (artesunate, artemether, β-arteether, *etc.*), and is also available as a drug itself (Bruxvoort *et al*, 2014). The absence of efficient vaccine against these diseases, the absence or the high toxicity of the few drugs against America and Africa and the emergence of chemoresistance against *Plasmodium falciparum* emphasize the necessity to produce new antiparasitic strategies.

Plasmodium falciparum has developed resistance to nearly all antimalarial drugs in current use, although the geographic distribution of resistance to any one particular drug varies greatly. In particular, Southeast Asia has a highly variable distribution of falciparum drug resistance; some areas have a high prevalence of complete resistance to multiple drugs, while elsewhere there is a spectrum of sensitivity to various drugs. The change in the malaria burden trend is a result of the interventions (such as, mechanical forms of protection such as the use of insecticide treated nets, administration of combination drug therapy regimens such as Arthemisin Combination Therapy"s (ACTs) (MoHSS, 2009) and Indoor residual spraying) implemented by the malaria campaign "Wipe out Malaria", established by the Ministry of Health and Social services (MoHSS) (WHO, 2010).Current limitation of vaccine, vector control and increasing resistance of malaria parasite for existing drug, necessitated the continuous need for the search of new antimalarial agent and target. Potential target for chemotherapy include malaria parasite protease which are required for the rupture and subsequent reinvasion of erythrocytes by merozoite-stage of *Plasmodium* activities possibly to breach the erythrocyte cytoskeleton complex network of protein (Mckerrow *et al.*, 2001).They are also needed for the degradation of haemoglobin by intra-erthrotrophozoite necessary for the growth of erythrocyte malaria parasite, apparently to

provide free amino acid for parasite protein synthesis (Rosenthal *et al.*,1994). The key measures for control and subsequent elimination of malaria are prevention, vector control, and treatment. Even though many measures need to be used simultaneously in the fight against malaria, antimalarial drugs remain critical in the control and eventual elimination of malaria parasite (White, 2008).

Most communities in Africa continents make use of plant parts either as decoction extract, infusion, or tinctures for the treatment of various types of diseases that infect them (Boadu and Asase, 2017). Plants of medical importance have an essential role in the development of new drugs and ensuring an efficient healthcare system of many nations including Nigeria. According to Newman *et al.* (2000), at least 119 chemical substances originating from plants can be considered as important drugs for the treatment of various ailments across many nations. Secondary metabolites of plants (phytochemicals) with previously unknown pharmacological activities have been extensively investigated as medicinal agents (Sofowora, 1993). These secondary metabolites differ from plant to plant and include such examples as: anthraquinones, flavonoids, glycosides, saponins, tannins etc. Plants also contain other compounds such as morphine, atropine, codeine, steroids, lactones and volatile oils, which possess medical values for the treatment of different diseases (Sofowora, 1993).

Jatropha curcas commonly known as physic plant or purging nut or big purginant, is a drought resistant large shrub, belonging to the family Euphorbiaceae. The plant is commonly known as Lapalapa in Yoruba, Wuluidu in Igbo and Bini da zugu in Hausa. *Jatropha* is a hearty multipurpose shrub type tree that grows wildly in Nigeria with little or no maintenance. The oil-rich seeds are very attractive feed stock for biofuel production (Jongschaap *et al*., 2007). The

kernel consists of about 60% oil which can be transformed through esterification into biofuel. The green energy of the plant can be used to power various machines while the biofuel mix was reported as being more efficient and burn less fuel in total than the conventional one. It burn with clear smoke-free flame. Additionally, the plant is cherished for its medicinal value and the cake can be used as livestock feed if properly processed. There are different species of Jatropha but *J. curcas* is one of commonest species of plants found in Nigeria. Traditionally,*Jatropha curcas* is used for the treatment of fever, mouth infections, jaundice, guinea worm, sores and joint rheumatism (Villegas *et al.,* 1997;Van den Berg *et al.,* 2010). Various scientific works have authenticated the numerous medicinal properties of *J. curcas,*Sarkiyayi *et al.* (2016) reported the antiplasmodial and hepatoprotective effect of aqueous stem bark extract of *Jatropha curcas* on *Plasmodium beighei* infected mice. The ethanol leaf extract was reported by Ehsaan *et al.* (2011) to possess antioxidant and anti-inflammatory effects, while the hepatoprotective effect of the methanol leaf extract on cadmium induced toxicity was demonstrated by Adejumobi *et al.* (2015) using rabbits. Ehsaan *et al.* (2011) reported the anti-cancer effect of the metanolic leaf extract of the plant. Studies also reported the presence of antibacterial agents in different parts of *J. curcas* (Mishra *et al.,* 2010)

1.2 Statement of Research Problem

The World Health Organization reported there are 228 million cases of malaria worldwide as at2019 which resulted in an estimated 405,000 to 855,000 deaths, the majority (93%) of which occurred in Africa (WHO, 2019). In its entirety, the economic impact of malaria has been estimated to cost Africa 12 billion U.S Dollars every year (Worrall *et al.,* 2005; Humphreys, 2010).

This cost include; cost of health care, working days lost due to sickness, days lost in education, decreased productivity due to cerebral damage associated with *Plasmodium falciparum* infection and loss of investment and tourism (Sachs and Malany, 2002: Greenwood *et al.,* 2005). In addition to the cost of antimalaria drugs, the huge health problem of malaria is exacerbated by the alarming ability of this *P. falciparum* to develop resistance to drugs such as quinine, antifolate combination drugs and artemisinin and its derivatives. Clinical resistance to these combinations has been reported (Noedl *et al*., 2008), suggesting that *P. falciparum* have already developed the ability to grow in the presence of these anti-malaria, which strongly suggests for the need to further research into new anti-malaria drugs. The discovery of new and effective antimalaria drugs based on new mechanisms of action or with new structures, is urgently needed to overcome the problem of rapid emergence of drug resistance and achieve long-term clinical efficacy. Natural products (plants) have been a thriving source of discovery of new drugs due to their chemical diversity and ability to act on various biological targets. Also, the isolation of new bioactive compounds from medicinal plants based on ethnomedical and traditional data appears to be a very promising approach (Turschner *et al.*, 2009).

1.3 Justification

The ineffectiveness of chemotherapy constitutes the greatest threat to the control of malaria. Therefore, to overcome malaria, new knowledge, tools and products are urgently needed especially new drugs are of importance (Rasoanaivo *et al*., 2011). New drugs against *P. falciparum* are urgently needed and the traditional methods of malaria treatment could be a promising source of new antimalarial compounds (Benoit-Vical, 2005).The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used in the

world to prevent or to cure diseases, scientific evidence in terms of modern medicine is lacking in some cases.

The local populace widely uses *J. curcas* plants for the treatment of many ailments including malaria. However, there are no scientific bases for this treatment. Therefore, this research is designed to provide the scientific evidence for the use of the leave extract of *J. curcas* in the treatment of malaria and its safety in the medication.

1.4 Aim

To evaluate the *in vivo* anti-plasmodial effects of the aqueous and methanolic fractions of the plant leaf extract of *J. curcas* on *Plasmodium berghei* infected mice.

1.5 Objectives

The objectives of this study are to determine:

I. phytochemical constituents of the aqueous and methanolic leaf extract of *Jatropha curcas*
II. *in vivo* activity of the aqueous and active fractions of methanolic leaf extract of *J. curcas* against *Plasmodium berghei* infected mice
III. the effect of aqueous and active fractions of methanolic leaf extract of *J. curcas* on haematological parameters in Mice
IV. ameliorative activity of the aqueous and active fractions of methanolic leaf extract of *J. curcas* on the histology of heart, intestine, liver and kidney in mice infected with *P. berghei*

1.6 Research Hypotheses

I. Aqueous and methanolic extract of *Jatropha curcas* leaf does not have any phyto-constituents

II. Aqueous and active fractions of methanolic leaf extract of *J. curcas* are not effective against *Plasmodium berghei*

III. Aqueous and active fractions of methanolic leaf extract of *J. curcas* has no significant effect on haematological paramaters of the infected mice

IV. Aqueous and active fractions of methanolic leaf extract of *J. curcas* has no ameliorative activity on the vital organs (heart, intestine, liver and kidney) of infected mice

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria

Malaria infection is reported to have plaque human from pre-historic times and remains one disease with immense social cost, being responsible for unacceptable huge number of deaths. The term "Malarial" came into use in the 18th century from Italy where people associated malarial with bad air (Mala-Bad, Aria-Air). It is interesting to note that the treatment of the disease became first established in the middle of the seventeenth century before anything was known about its etiology and how the disease was transmitted. Malaria was formerly called Marsh-fever by the British, is an infectious disease caused by a parasite of the genus *Plasmodium* affecting mostly women and children in many parts of the world, especially sub-saharan Africa. This disease is characterized by recurrent symptoms of chills, fever, headache and pains in the joint, nausea, vomiting anemia, diarrhea, muscle pain, convulsion, coma, bloody stool, causing about 350-500 million infections worldwide leading to 1.3-3.0 million deaths annually.

Malaria is a vector borne infectious disease caused by protozoan, parasites from the phylum *Apicomplexa* (Heelan *et al*., 2002) and of genus *Plasmodium.* The genome of several plasmodium species Includes; *Plasmodium falciparum, Plasmodium knowlesi, Plasmodium vivax, Plasmodium berghei* and *Plasmodium yoelii.* Several species are found, of which only four cause malaria in human namely *P. vivax, P.malariae, P.ovale and P.falciparum. Plasmodium falciparum* is the most serious form of the disease as it is responsible for the high malarial mortality and morbidity rates in Africa and occurs predominantly in Nigeria. Malaria is the 3rd leading cause of death for children under five years worldwide, after pneumonia and diarrheal disease (USAID, 2013). Thirty countries in Sub-Saharan Africa account for 90% of global

malaria deaths (USAID, 2013). Nigeria, Democratic Republic of Congo (DRC), Ethiopia, and Uganda account for nearly 50% of the global malaria deaths (WHO, 2013). Malaria is the second leading cause of death from infectious diseases in Africa, after HIV/AIDS. Almost 1 out of 5 deaths of children under 5 years of age in Africa is due to malaria (WHO, 2010). In Nigeria malaria is a major public health problem, where it accounts for more cases and deaths than any other country, about 97% of Nigeria's population are at risk. The remaining 3% of the population live in the malaria free highlands.

There are an estimated 100 million malaria cases with over 300,000 deaths per year in the country. This compares with 215,000 deaths per year from AIDS. Malaria contributes to an estimated 11% of maternal mortality, it accounts for 60% of outpatient visits and 30% of hospitalizations among children under five years of age.Malaria has the greatest prevalence, close to 50% in children age 6 and below (WHO, 2010). According to Rosenthal (2003) resistance to existing antimalarial drugs is mostly seen in *P.falciparum*. All these species have genomes of about 25 megabases organized into 14 chromosomes consistent with earlier estimates. The chromosomes vary in length from 500 kilobases to 3.5 megabases and it is presumed that this is the pattern throughout the genus. The *Plasmodium* contains a degenerated chloroplast called an apicoplast. As a protest, the plasmodium is a eukaryote of the phylum *Apicomplexa*. Unusual characteristics of this organism in comparison to general eukaryotes include the *rhoptry, micronemes*, and polar rings near the apical end. According to the latest estimates, there were about 219 million cases of malaria in 2010 and an estimated 660,000 deaths. Malaria mortality rates have fallen by more than 25% globally since the year 2000 and by 33% in the WHO African Region. Most deaths occur among children living in Africa where a child dies every minute from malaria. Country level burden estimates available for 2010 show

that an estimated 80% of malaria deaths occur in 14 countries and about 80% cases occur in 17 countries (David *et al*., 2004). Survey shows that 90% of the world's cases of malaria occur in Sub-Saharan Africa. Nine out of ten cases of this disease occur in this region and record over one million deaths annually (World Malarial Report, 2005).

Malaria is a major public health problem in Nigeria. Every year, thousands of cases are reported from all over the country. It is the leading parasitic cause of morbidity and mortality in the tropics. One quarter of the world's population is at risk for malarial infection (Martens *et al*., 2002). The present global situation indicates a recent resurgence in the severity of the disease and that malaria could still be described as one of the most deadly diseases, with an annual incidence of 300-500 million clinically manifest cases and a death toll of 1-2 million people (Martins *et al.,* 2004). World Health Organization malaria report in 2008 puts the figure at 247 Million clinically manifest cases and a death toll of 881,000 people (Breiger *et al*., 2009). Mortality and morbidity due to malaria are a matter of great concern throughout the whole world, especially in the tropical and subtropical regions, even though casualty in children below the age of 5 years is very high, the disease affects all age groups (Bickii, 2000).

2.2 Transmission of Malaria

Malaria is transmitted through the bites of female anopheles mosquito that is looking for a blood meal and is infected with the malaria parasites; *Plasmodium* (Egan, 2001). The parasites enter the blood stream and travel to the liver, where they multiply. When they re-emerge into the blood, symptoms appear. By the time a patient shows symptoms, the parasites have reproduced very rapidly, clogging blood vessels and rupturing blood cells. Malaria is also transmitted from mother to unborn baby (i.e. congenitally), by blood transfusion, malaria corresponds with the

rainy season which provides breeding sites for the Anopheles vector. The *Plasmodium* parasite is only carried by female *Anopheles* mosquitoes in warm, humid and wet climates but disappears over winter. Larvae control is therefore, imperative in the control of this epidemic disease. Other factors that contributes to the increase of malaria incidences include travelling to and from epidemic areas, the accidental imports of the malaria vectors (a phenomenon known as "airport malaria") and the increase in agricultural activities (MoHSS, 2005).

2.3 Life Cycle of Malaria Parasite

The causative agents of malaria are*P. ovale, P.falciparum, P. malariae and P. vivax* and they all have a complex life cycle involving two hosts: the human and the Anopheline mosquito. Whenever an infected mosquito has a blood meal, it injects saliva containing the parasite (in the form of sporozoites) into the pierced skin serving as some kind of anesthesia. The sporozoites now trail through the bloodstream of the victim and infect the liver. Growth and division in the liver for the human malaria parasites take from approximately 6 to 15 days depending on the species: approximately 6 days for *P. falciparum*, 10 days for *P. vivax*, and 15 days for *P. ovale* and *P. malariae*. In the liver, the sporozoites reside in the parenchyma cells (Machado*et al.*, 2001) and asexual development or schizogony takes place. The parenchymal cells transform into a circular shape and start to divide, producing many merozoites, which are released into the bloodstream upon rupturing of the hepatocytes. Once the bloodstream they infect the red blood cells (RBCs) and start to multiply. At the end of the pre-erythorcytic cycle, thousands of merozoites are released into the blood flowing through the sinusoids and within 15 to 20 seconds, attach to and invade erythrocytes.

In *P. vivax* and *P. ovale*, some of the sporozoites appear to develop for about 24 hours before becoming dormant as a hypnozoite stage. This form can remain as such for months and even

years until reactivated to complete the life cycle, releasing merozoites into the blood to precipitate a relapse infection. The erythrocytic life cycle responsible for all clinical manifestations of malaria, begins when free merozoites invade erythrocytes. The intraerythrocytic parasites develop from small ring-stage organisms to larger, more metabolically active trophozoites and then to multinucleated schizonts. The erythrocytic cycle is completed when mature schizonts rupture erythrocytes, releasing numerous invasive merozoites.

According to Mechado (2001) the rest of the merozoites grow as trophozoites (i.e. the ring forms), increase in size, divides mature into schizonts. Merozoites are formed from the schizonts as a result of erythrocytic schizogony. Within 48-72 hours the 40 infected merozoites undergo gametocytogenesis (Inga and Slems, 2002) and develop into gametocytes (i.e. macro and microgametocytes) which can only mature outside the human body. When a mosquito takes a blood meal from an infected person, both the merozoites and gametocytes travels to the stomach/gut of the mosquito. The merozoites are digested, whereas the gametes develop into a zygote and then into an ookinete (fertilized egg). The ookinete matures into an ocyst giving rise to sporozoites. These sporozoites, when released, travel to the salivary gland. When the female Anopheles bites the next victim, the cycle starts all over again.

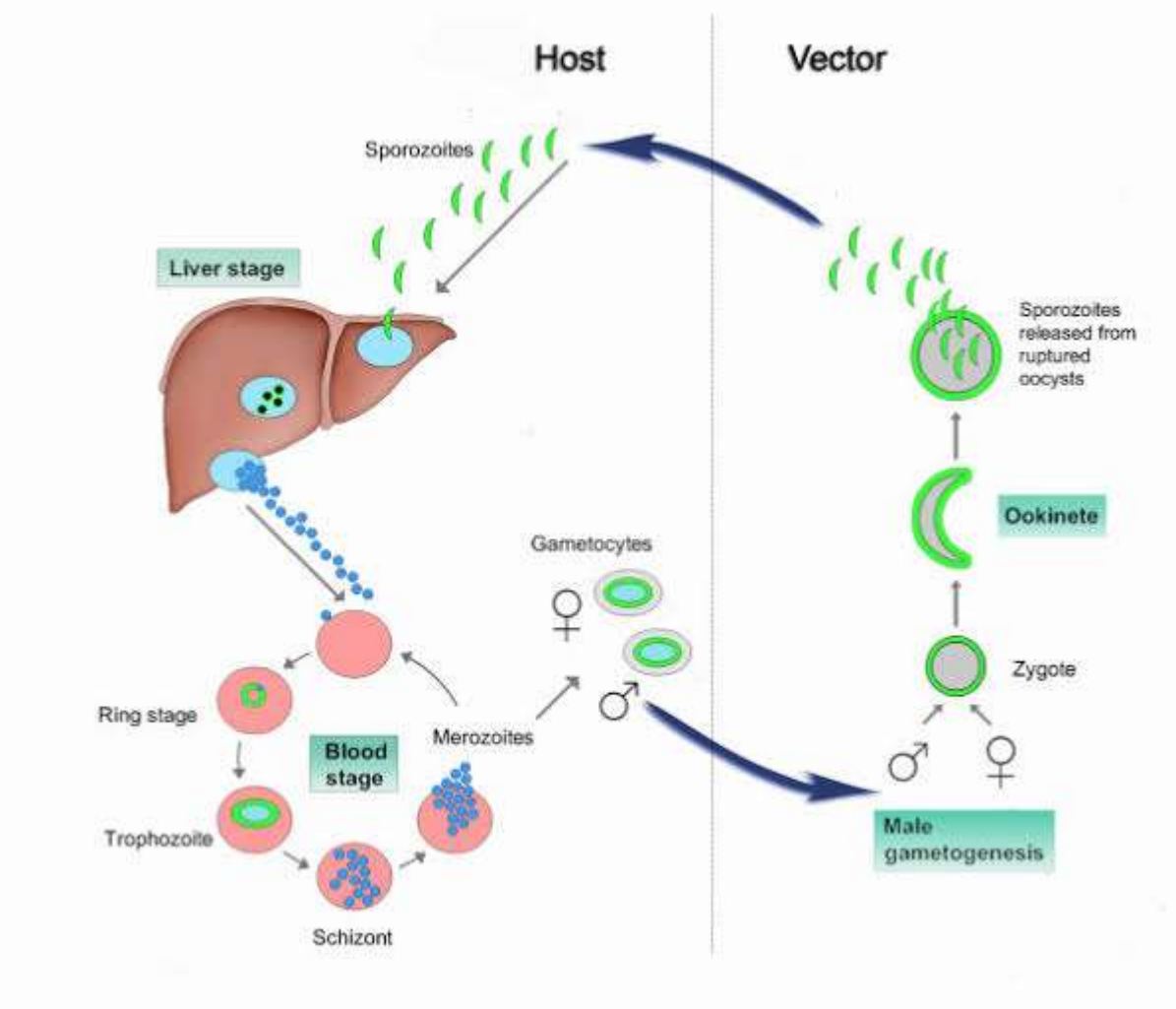

Fig. 2.1: Life Cycle of *Plasmodium* Species **Source: Ksenija *et al.*, (2011)**

2.4 Epidemiology of Malaria

Malaria is one of the most widespread diseases in the world and is endemic through tropical and subtropical regions. International communities have invested in malaria control over the past 20 years. However, malaria still remains the most widespread blood parasite disease in the world. Malaria endemic areas involve 108 countries inhabited by roughly 3 billion people, which represents over 40% of world population. *P. vivax* and *P. falciparum* are known to cause 80-95% of all malaria cases worldwide (Wesolowski *et al.*, 2015)

According to WHO, (2017) Malaria transmission occurs in five WHO regions. Globally, an estimated 3.2 billion people in 95 countries and territories are at risk of being infected with malaria and developing disease and 1.2 billion are at high risk (>1 in 1000 chance of getting malaria in a year). According to the World Malaria Report (2015), there were 214 million cases of malaria globally in 2015 (uncertainty range 149–303 million) and 438 000 malaria deaths (range 236 000–635 000), representing a decrease in malaria cases and deaths of 37% and 60% respectively since 2000, respectively. The burden was heaviest in the WHO African Region, where an estimated 90% of all malaria deaths occurred, and in children aged under 5 years, who accounted for more than two thirds of all deaths.

According to the World Malaria Report (2016), the rate of new malaria cases fell by 21% globally between 2010 and 2015. Malaria death rates fell by 29% in the same 5-year period. In sub-Saharan Africa, case incidence and death rates fell by 21% and 31%, respectively. Other regions have made substantial gains in their malaria responses, but the disease remains a major public health threat. In 2015, the global tally of malaria reached 429 000 malaria deaths and 212 million new cases. One child died from malaria every 2 minutes (WHO, 2017).

In 2015, it is estimated that 7 of the 43 countries in sub-Saharan Africa with malaria transmission had more than 25% of their population infected with malaria parasites (Burkina Faso, Cameroon, Equatorial Guinea, Guinea, Mali, Sierra Leone and Togo); this number has decreased from 12 countries in 2010 (WHO, 2016). In 2015 out of 182,200,000 million malaria cases in Nigeria, 76% of the population had high transmission of malaria, 24% of the population had low transmission with no free malaria cases. Hundred percent of the circulating parasite is *Plasmodium falciparum* which is transmitted by 6 anopheles species*(An gambiae, An. Funestus, An. Arbiensis, An. Moucheti, An. Melas, and An. nili*). The prevalence of *P. falciparum* in

Nigeria is reported to be high in Kaduna, Kano, Kebbi (North-west), Cross-river and Akwa-Ibom (South-south), South-west region and Kwara, Niger (North-central) region (WHO, 2017).

2.5 Prevalence of Malaria

2.5.1 Prevalence of malaria in Africa

Most clinical events attributable to *P. falcipurum* were concentrated in the African region (70%). The World Health Organization suggested that there were 273 million clinical attacks of malaria worldwide in 1998 and that 90% of the global disease incidence is born in Africa. It is estimated that an individual receives 40-120 infective mosquito bites per year, compared to only 2 per year in other regions. Bearing the figures for Africa in mind, it seems ridiculous that 100% global eradication was envisaged from a policy, which effectively ignored Africa other than for a couple of pilot schemes (Woodruff *et al.*, 1987). Because the African region has a notoriously weak system of reporting infectious diseases, epidemiological evidence from carefully conducted prospective active case-detection studies of malaria morbidity, disability and mortality in populations leaving under different transmission intensity risks have been compiled to estimate the disease burden (Hay *et al*, 2004, Robert *et al*., 2005).

Mapping malaria risk in Africa (MARA) database findings on climate suitability for stable malaria transmission estimated that 200 million people (24.6% of the total African population) currently live in urban settings where they are at risk of contracting the disease. This is cause for great concern in that the surface area covered by these urban settings is approximately 1.1-1.6% of the total African surface. It was also estimated that an annual incidence of 24.8–103.2 million cases of clinical malaria attacks are among urban dwellers in Africa. These figures translate to 6-28% of the estimated global annual incidence of the disease (Snow *et al.*, 2003). It appears that urban malaria control will be more cost-effective than in rural areas, but research is needed to

confirm that this malaria epidemics have been on the increase during the last 10 years, which results from special interactions between vectors, parasites, humans, and various environmental and anthropogenic determinants. The explosiveness of malaria epidemics always strains the capacity of health facilities, causing case fatality rates to increase five-fold or more during outbreaks. People of all ages remain susceptible to the full range of clinical effects (Joel *et al.*, 2014).

2.5.2 Prevalence of malaria in Nigeria

Malaria is the commonest cause of hospital attendance in all age groups in all parts of Nigeria. It is also one of the four commonest causes of childhood mortality in the country, the other three being acute respiratory infection (pneumonia), diarrhea and measles. It is estimated that 50 percent of the population has at least one episode of malaria each year while children under five years of age have an average of 3 to 4 attacks in a year. The disease is characterized by a stable perpetual transmission in all parts of the country. Federal Ministry of Health (FMOH) (2017) reported that the rate of malaria transmission are high in the wet season compared to the infection rate in the dry season.

2.6 Diagnostics and Clinical Symptoms

The severity of the disease varies from mild asymptotic infection to the critical complicated disease which causes death. Common symptoms include periodic flu-like symptoms such as fever, chills, sweating, muscle aches and headaches. Other symptoms include nausea, vomiting, coughing, diarrhea, abdominal pain, myalgia (limbs and back), loss of appetite, orthosatic hypotension, jaundice (yellowing of skin and eye white), aneamia, liver or kidney failure, enlarged liver and spleen and convulsions. Severe malaria is regarded as a multisystem disorder and includes cerebral malaria and severe malarial anemia (Weatherall *et al.*, 2002). Hans (2009)

claims severe malaria delirium, metabolic acidosis and multi-organ dysfunction which may occur, if untreated could result in coma and eventually death. Cyclical symptoms such as fever, seizures, chills and anemia are said to correspond with the erythrocytic stage of the *Plasmodium* life cycle during which merozoites are released into the bloodstream (Rayner *et al*., 2005).

Together with this release, there is also a deposit of parasitic waste products and debris, which is believed to give rise to the malaria paroxysm, i.e. the sequenced events of shaking chills, fever and sweating. Symptoms for complicated or severe malaria includes seizures, altered state of consciousness (or coma), excessive sleepiness, prostration (feelings of helplessness), respiratory distress, inability to ingest any fluids, bleeding problems, jaundice, the absence of urine and the dark coloration of urine if there is any, on the other hand maintain that splenomegaly and anemia are the major/principal clinical symptoms of malaria. The latter is caused by destruction of RBCs and the simultaneous loss of hemoglobin or by the removal of the infected erythrocytes as an immune response. Anemia may also be caused by the ceased production of RBCs due to the bone marrow suppression.

2.7 Complications of Malaria

2.7.1 Cerebral malaria

Cerebral malaria is an acute febrile and mainly diffuse encephalopathy, occurring in a patient infected with *P.falciparum*. The World Health Organization Malaria Action Programme proposed three criteria for the diagnosis of cerebral malaria: unarousable coma, exclusion of other encephalopathies and confirmation of *P.falciparum* infection (Schmutzhard, 1998). The pathogenesis of cerebral malaria is heterogeneous and the neurological complications are often

part of a multisystem dysfunction. The clinical presentation and pathophysiology differs between adults and children (Newton *et al.*,2000).

Cerebral malaria is the most severe and common complication affecting up to 7% of all *P.falciparum* malaria cases and with mortality rates up to 50%. Initially patients often complain of nonspecific symptoms, even days before the onset of the paroxysm: malaise, headache, myalgia and fatigue are easily mistaken for beginning viral illness. Children usually experience an abrupt onset, frequently with hyperpyrexia, headache, restlessness and vomiting (Schmutzhard, 1998). Within hours but sometimes much slower features of CNS involvement set in. In malignant falciparum malaria however an asynchronous cycle of parasite multiplication leads to continuous, remittent or irregular. Unarousable coma may be preceded by severe headache, confusion, drowsiness, and in many instances convulsions. Meningeal irritation is rare as extra pyramidal and cerebeller signs, retinal hemorrhage, and exudates are infrequent. Hyper pyrexia, splenomegaly are frequently physical findings. Hepatomegaly is less often observed. In advanced disease, severe anemia, icterus, renal failure, acute pulmonary edema, heart failure, bleeding tendencies, spontaneous hemorrhages and hypoglycemia complicate the course of *P. falciparum* infection. In a series of 66 Tanzanian children, 73% presented with unarousable coma, 15% had an organic psycho syndrome with confusion, disorientation and restlessness. Almost a quarter showed localizing signs; two-thirds of the children had generalized rarely focal seizures. In 52 of 65 of Malawian children presented with convulsions (Schmutzhard, 1998). A cross sectional study was carried out to investigate clinical features and outcome of cerebral malaria in Indian rural area. Of the 56 cases positive for malarial parasite on peripheral smear (PS) 4.3% were *P.vivax* and 2.8 % *P.falciparum.*15 patients fulfilled the criteria for a study, of which 7 were definite cerebral malaria (DCM) and 8 belong to probable cerebral malaria (PCM)

group. 12(80%) were in school going age and male: female was 2:1. All patients presented with fever and CNS involvement, 66.6% had convulsion, 7 developed coma and anemia was seen in 66% but only 20% required blood transfusion (Kamble *et al.*, 2002).

2.7.2 Hypoglycemia

Hypoglycemia is an important complication of severe malaria. It is associated with poor prognosis particularly in children and pregnant women (White, 2005).

2.7.3 Lactic acidosis

Lactic acidosis commonly co-exists with hypoglycemia in malaria patients. It is an important contributor to death from severe malaria. The prognosis of lactic acidosis is poor (White, 2005). The most common clinical sign, deep breathing, occurs in nearly two-thirds of patients with acidosis. Acidaemia and hyperlactatemia may also be present (Taylor and Molyneux, 2002).

2.7.4 Pulmonary edema

Adults with *falciparum* malaria may develop noncardiogenic pulmonary edema even after several days of antimalarial therapy. The pathogenesis is unclear. The mortality is >80% (White, 2005).

2.7.5 Renal impairment

Renal impairment is more common among adults then children with severe falciparum malaria. It may be due to erythrocyte sequestration interfering with renal microcirculatory flow and metabolism (White, 2005).

2.7.6 Hematologic abnormalities

Common hematologic abnormalities are thrombocytopenia (platelet count<150×109/L) occurs in up to 70% of patients and anemia in 25% patient. The leukocyte count is normal or low. Leukocytosis is seen in less than 5% of cases and is poor prognostic factor (Suh *et al.*, 2004).

2.7.7 Liver dysfunction

Jaundice is more common among adults than children and results from: hemolysis, hepatocyte injury and cholestasis. Liver dysfunction carries a poor prognosis (White, 2005).

2.7.8 Hyper reactive malarial splenomegaly

It occurs infrequently and is attributed to an abnormal immune response to repeated malarial infections. The condition is associated with a very enlarged spleen, abnormal immunologic findings, anemia, and a susceptibility to other infections such as skin or respiratory infections (CDC, 2004).

2.7.9 Nephrotic syndrome

A chronic and severe kidney disease can result from chronic or repeated infections with *P. malariae.* This condition is an indication of poor prognosis (CDC, 2004).

2.7.10 Black water fever

Is a manifestation of *falciparum* malaria occurring in previously infected subjects and is characterized by sudden intravascular hemolysis followed by fever and hemoglobinuria. It is associated with infection by *Plasmodium falciparum*, most commonly observed among the non-immune (non-indigenous) individuals who have resided in malaria endemic countries previously for 6 months to 1 year and have had inadequate doses of quinine for both suppressive prophylaxis and treatment of repeated clinical attacks. In these cases quinine often act as a precipitating factor.

Other factors that have been known to precipitate an attack of black water fever are: cold, exposure to the sun, fatigue, trauma, pregnancy and parturition and X-ray treatment of the spleen (Chaterjee, 2006).

2.7.11 Relapse malaria

In *P. vivax* and *P. ovale* infections, patients having recovered from the first episode of illness may suffer several additional attacks (relapses) after months or even years without symptoms. Relapses occur because *P. vivax* and *P.ovale* have dormant liver stage parasites (hypnozoites) that may reactivate. Treatment to reduce the chance of such relapses is available should follow treatment of the first attack (CDC, 2004).

2.8 Pathogenesis of Malaria

2.8.1 Virulence factors

Plasmodium falciparum is one of the world's most devastating pathogen. It has an astonishing array of sequences and genes that play key roles in pathogenesis and immune evasion. Several factors such as: PfEMP1, rifin protein, stevor antigen, hemozoin, Glycosylphosphatidylinositol act as virulence factors in the pathogenesis of malaria.

2.8.2 *Plasmodium falciparum* erythrocyte membrane protein 1(PfEMP1)

Virulence of *Plasmodium falciparum* is associated with the expression of variant surface antigens designated PfEMP1 that are encoded by a family of var genes (Lavstsen, 2005). These PfEMP1 proteins are high molecular weight proteins are transported to the surface of the infected red cell, where they have been demonstrated via CD36 and to uninfected red cells via complement receptor1 and heparan sulfates. Indirect evidence also suggests that PfEMP1 is the ligand that binds to intercellular adhesion molecule1 (Kyes *et al*., 1999).

PfEMP1 also undergoes cloanal antigenic variation, with variant forms differing both antigenically and adhesion characteristics.The ability to adhere to different host endothelial receptors may also determine the virulence of *P.falciparum* (Hayward *et al*., 1999).

2.8.3 Rifin protein

In the genome sequence of *Plasmodium falciparum*, other unique multicopy gene families have been identified. The largest of which belongs to the *rif*(repetitive interspersed family) gene family (Abdel- Latif *et al*., 2002). This gene family encodes clonally variant proteins (rifins) that are expressed on the infected red cell. Their high copy number, sequence variability and red cell surface location indicate an important role for rifins in malaria host-parasite interaction (Keys *et al.*, 1999).

2.8.4 Stevor antigen

The third family of variant proteins comprises stevor antigens. Although stevor genes are located in the tandem with *rif*and var genes, they seem to be much more conserved among strains than the *Rif* and var genes. The stevor genes (30 to 40 copies per haploid genome) have, a two-exon structure similarly to that of rif genes and code for 30 to 40 kDa proteins with a rather short intracellular domain that are expressed over a brief period by mature trophozoites and possibly by sporozoites and gametocytes as well. Their biological function is unknown (Ferreira, 2004).

2.8.5 Others

The hemozoin (malaria pigment) has been implicated in the modulation of immune responses during malaria infection (Coban *et al*., 2002).Glycosylphosphatidylinositols are the anchor molecules of some membrane proteins of plasmodium species have also been implicated in the induction of TNFá and IL-1 during malaria infection (Souza *et al*., 2002; Angulo, 2002).

2.9 Virulence Events

2.9.1Cytoadherence

During the asexual cycle of malarial parasites which occurs within the RBCs. The infected RBCs adhere to the endothelial cells through electron-dense knobs on the RBCs. Several cell-surface molecules have been identified as potential receptors for RBC binding (Mackintosh *et al*., 2004). These include: Thrombospondin, CD36, ICAM-1, vascular cell adhesion molecule1 (VCAM1), E selectin, P-selectin, chondroitin sulfate A, áVâ3-integrin and platelet endothelial cell adhesion molecule. Adherence of *P. falciparum* infected erythrocytes to the endothelium of post capillary venules assists the parasite in avoiding splenic clearance and promotes sequestration in organs such as the brain and placenta (Reeder *et al*., 1999).

2.9.2 Sequestration

The process whereby erythrocytes containing mature forms of *P. falciparum* adhere to microvascular endothelium and thus disappear from the circulation is known as sequestration. It occurs predominantly in the venules of vital organs. It is not distributed uniformly throughout the body, being greatest in the brain, heart, eyes, liver, kidneys, intestine, adipose tissue and least in the skin (White, 2003). Sequestration occurs principally during the second half of the intra-erythrocytic asexual growth phase of the parasite, following adherence (Mackintosh *et al*., 2004). Sequestration is the characteristic feature of infection with *P.falciparum*. This is the process involving the accumulation of large numbers of parasitized erythrocytes in various organs. It is mediated by adhesive interactions between parasite ligands on the surface of the infected erythrocytes (IEs) and host molecules present on microvascular endothelium (Beeson *et al*., 1999). Sequestration is facilitated by the expression of knob like productions under the surface of the infected RBC membrane (Hayward *etal*., 1999).

2.9.3 Rosetting

Erythrocytes containing mature parasites, in addition to adhering to the endothelial cells and syncytiotrophoblast, mature-stage parasitized RBCs (pRBCs) can also adhere to the un-infected RBCs. This process leads to the formation of rosettes when suspensions of parasitized erythrocytes are viewed under the microscope (White, 2003).

2.9.4 Deformability/RBC changes

As the parasite matures inside the erythrocyte, the normally flexible biconcave disc becomes progressively more spherical and rigid. The deformability results from reduced membrane fluidity, increasing sphericity and the enlarging and relatively rigid intra-erythrocytic parasite (White, 2003).The membrane fluidity of the pRBCs is much lower than that of uninfected cells, and this renders pRBCs less flexible, more liable to damage in the circulation and more susceptible to spleenic clearance. However, uninfected RBCs also show alterations in their rheological characteristics during malaria which may also render them more susceptible to damage or splenic clearance (Warrell *et al.*, 2002).

The differences between parasite infected and uninfected RBCs are great such as: mechanical properties of pRBCs are changed. The membrane is less flexible, which makes it difficult for the cell to pass through the microvasculature, parasite nutrients: carbohydrates, amino acids and purine bases are transported into the cells. Furthermore, some pRBCs membrane components are digested or modified. Most interestingly several parasite derived polypeptides, including PfMP1 and rosettins/rifins are inserted into and then protrude from the membrane. All these parasite-derived polypeptides significantly change the nature of the RBC membrane (Chen *et al*., 2000).

2.10 Factors Involved in Pathogenesis

2.10.1 Parasite factors

2.10.1.1 *Parasite adherent ligands*

At least five parasite-derived proteins are associated with the cell membrane of an infected erythrocyte at various stages of the developmental cycle. Three of the malarial proteins are associated with knobs, PfEMP1, PfEMP2 and PfHRP1. Of these, PfEMP1 is the only protein that extends beyond the cell surface to mediate cytoadherence, whereas PfEMP2 and PfHRP1 remain on the internal surface of the erythrocyte membrane in association with electron-dense material. Two other parasite proteins, ring-infected erythrocyte surface antigen (RESA) and PfHRP2, are associated with the erythrocyte membrane but are not localized specifically to the knobs. PfHRP2 is secreted into the circulation and is currently being exploited for diagnostic assays (Lavstsen, 2005).

2.10.1.2*Toxins*

Fatalities due to malaria are associated systemic and organ-specific inflammation initiated by parasite toxins. Recent studies show that glycosylphosphatidylinositol (GPI) functions as the dominant parasite toxin in the context of infection. GPI also serve as membrane anchors for several of the most important surface antigens of parasite invasive stages (Delorenzi *et al*., 2002).

2.10.2 Host factors

2.10.2.1 *Endothelial receptors*

The stage and host cell specificity of cytoadherence suggests that the process involves specific parasite or host ligands expressed on the surface of IRBC and vascular endothelium. A number

of endothelial receptor molecules have been identified, based on their ability to support the adhesion (Lavstsen, 2005).

2.10.2.2 *Thrombospondin (TSP)*

This was the first described endothelial receptor for infected RBC (IRBC). IRBC adhere to immobilized TSP in a dose-dependent manner. Subsequent investigations showed that, although TSP may contribute to cytoadherence, it is not sufficient to mediate the process by itself (Ho and White, 1999).

2.10.2.3 *CD36*

The second receptor molecule to be implicated in cytoadherence was CD36. CD36 is found on monocytes, endothelial cells, platelets, and erythroblasts. It has been shown that IRBC interact with sites on the CD36 molecule that are distinct from the binding sites of TSP. The interaction of IRBC with CD36 expressed on monocytes leads to a respiratory burst, with the production of oxidative metabolites that are toxic to intraerythrocytic parasites (Ho and White, 1999).

2.10.2.4 *Intercellular adhesion molecule-1 (ICAM-1)*

This is a glycoprotein and plays a central role in the generation of an immune response. It acts as a receptor for IRBC selected on human umbilical vein endothelium. It is distributed widely on venular endothelium, where it has been shown to be crucial for neutrophil adhesion before the transmigration of these cells into an inflammatory focus. ICAM-1 expression on endothelial cells can be upregulated by the proinflammatory cytokines, tumor necrosis factor (TNF), interleukin-1 (IL-1), and interferon (IFN) (Ho and White, 1999).

2.11 Pathophysiology

Clinical illness is caused by the erythrocytic stage of the parasite. No disease is associated with sporozoites, the developing liver stage of the parasite, the merozoites released from the liver, or

gametocytes. When an infected female anopheles mosquito takes blood meal from human host,sporozoites are inoculated in human blood. Within half an hour the sporozoites attach to and invade liver cell (Todd *et al*., 2004).

The sporozoites bind to the hepatocyte receptor for the serum proteins, thrombospondin and properdin. Within the liver cell malaria parasites multiply rapidly, as many as 30,000 merozoites are released, when hepatocyte ruptures. In case of *P.vivax* and *P.ovale*, hypnozoites (latent form) are formed. Which cause relapse malaria in later (McAdam and Sharpe, 2004). The preerythrocytic stage of infection produces minimal histopathologic changes. No detectable symptoms or functional disturbances seen in host (Warrell, 2002). After entry into the blood stream, merozoites rapidly invade erythrocytes. Attachment with RBC is mediated via specific surface receptor present on the erythrocyte.

The receptor for *P. vivax* is related to the Duffy blood-group antigen Fya or Fyb (White, 2005). In case of *P. falciparum* merozoites bind by a parasite lectinlike molecule to sialic residues on glycophorin molecules on the surface of the red blood cells and invade (McAdam and Sharpe, 2004). It is observed that *P. falciparum* invades erythrocytes of all ages, *P.vivax* and *ovale* invades young RBC whereas *P.malariae* invades normoblasts (Todd *et al.*, 2005). Clinical illness in human begins with the direct effects of red cells invasion and destruction by the asexual stage of parasite and the hosts reaction. After invading an erythrocyte, the growing parasite progressively consumes and degrades intracellular protein, principally hemoglobin; the potentially toxic heme is polymerized to biologically inert hemozoin, or malaria pigment. The parasite also alters the red cell membrane by changing its transport properties, exposing cryptic surface antigens and inserting new parasite derived proteins (White, 2005).

Studies suggest that the released factors and altered red cell surface membrane causes stimulation of the reticulo-endothelial system, changes in regional blood flow and vascular endothelium. Which causes release of cytokines such as: TNFá and interlukins, reactive oxygen intermediates, and other cellular products and play a prominent role in pathogenesis. These are responsible for febrile paroxysms, headache, pain and prostration, which are the most familiar and consistent symptoms of an acute malaria attack (Warrelll, 2002). The life cycles of the plasmodium species are similar, although *P. plasmodium* differs in ways that contribute to its greater virulence. Hypoglycemia is a recognized complication of severe malaria. One of the several causes of hypoglycemia is quinine induced hyperinsulinemia, which results in reduced hepatic gluconeogenesis and increased uptake of glucose by peripheral tissues. Other causes include: increased metabolism of glucose by parasite, increased host tissue metabolism, depletion of carbohydrate stores by starvation and malnutrition and glucose malabsorption from reduced splanchnic blood flow (Shulman, 2002). Pulmonary edema is the most dreaded complication of malaria may develop at any stage of the disease (Warrell, 2002). In these cases, the mechanism is thought to involve leukocyte and complement mediated endothelial damage, resulting in leaky pulmonary capillaries (Shulman, 2002).

Lactic acidosis is caused by the combination of anaerobic glycolysis in tissues where sequestered parasites interfere with microcirculatory flow, lactate production by the parasites and a failure of hepatic and renal lactate clearance (White, 2005). The pathogenesis of renal failure is unclear but may be related to erythrocyte sequestration interfering with renal microcirculatory flow and metabolism. Clinically and pathologically, this syndrome manifests as acute tubular necrosis (White, 2005). The pathogenesis of anemia is complex and undoubtedly involves multiple processes relating to both the destruction of erythrocytes and their reduced

production(Mackintosh *et al*., 2004). Primary cause of anemia is infected RBCs are directly destroyed by infecting parasites. However, other processes such as: dyserythropoesis, enhanced splenic clearance and even blood loss, also contribute to anemia (Warrell *etal.*, 2002). It is also suggests that during *P. falciparum* infection also causes suppression of bone marrow response to erythropoetin (Mackintosh *et al.*, 2004).

Thrombocytopenia is a common finding in falciparum and vivax malarias. Platelet survival is reduced to 2-4 days in severe falciparum malaria. Enhanced splenic uptake or sequestration may also contribute to thrombocytopenia. In patients with DIC; platelets may be removed from the circulation at sites of fibrin deposition (Warrell, 2002). Mild leukopenia has been described in uncomplicated malaria, but a neutrophilic leukocytosis is an important abnormality in patients with severe falciparum malaria. This is associated with a bad prognosis (Warrell, 2002). Hyper reactive malarial splenomegaly is seen in older children and adults in areas where malaria is hyper endemic. It is associated with an exaggerated immune response to repeated malaria infection, and is characterized by anemia, massive splenomegaly and elevated IgM levels. In tropical splenomegaly syndrome **(**TSS) malaria parasites are scanty or absent. TSS usually responds to prolonged treatment with prophylactic antimalarial drugs (Finch *et al*., 2005). Chronic or repeated infections with *P.malariae* may cause soluble immune complex injury to the renal glomeruli, resulting in the nephrotic syndrome. Malaria nephropathy usually responds poorly to treatment with either antimalarial agents or glucocorticoides and cytotoxic drugs (White, 2005).

2.12 Immunity to Malaria

Both innate and acquired immunity play an important role in malaria. Initially the host responds to plasmodium infection by activating non-specific defense mechanism (White, 2005).

2.12.1 Innate immunity

Innate immunity is thought to play a crucial role in clearing parasite from infected host. Most of the elimination occurs in the spleen under normal circumstances. Although the liver has been shown to function as an alternative clearing site. Within the spleen this task is apparently assumed by macrophages of the red pulp (Angulo and Fresno, 2002). Several inherited alterations in red blood cell give partial immunity to malaria in some population. It seems to cleared that Sickle cell disease, G6PD deficiency, thalassemia and other hemoglobinopathies provide some protection against lethal levels of falciparum infection. People who are heterozygous for the sickle cell trait (HbS), they are less likely to die from *P. falciparum* infection.

HbS trait causes the parasites to grow poorly or die at low oxygen concentrations, perhaps because of low potassium levels caused by potassium efflux from red blood cells on hemoglobin sickling. HbC protects against severe malaria by reducing parasite proliferation. Individuals with the HLA B-53 are resistant to *Plasmodium falciparum,* because HLA B-53 presents liver stage-specific antigens to cytotoxic T cells (McAdam and Sharpe, 2004). Another red cell deformity is ovalocytosis, which confers reduced risk of infection with *P. falciparum* and *P.vivax* malaria. Due to the absence of the Duffy antigen (Fy Fy) people become resistant to *P.vivax* infection, because this antigen acts as a receptor for *P.vivax* malaria (Carter and Mendis, 2002).

2.12.2 Acquired immunity

Both cellular and humoral immunity is involved in the process of acquired immunity (WHO, 1997). Individuals who are repeatedly exposed to malaria develop antibodies against sporozoites, liver stage, blood stage and sexual stage of parasite antigens. It is thought these antibodies are

responsible for the decreased susceptibility to malaria infection and disease (Krause, 2000) Passive transferred IgG from mother to fetus contributes to the relative protection of infants from severe malaria in the first months of life (White, 2005). Another type of acquired immunity has been observed that appears to depend upon the presence of low-level parasitemia that somehow inhibits new infections on maintains the infection at a nonsymptomatic level. This is called premunition. This type of immunity is soon lost after the parasites disappear from the blood (White,2005).

2.12.3 Immunity to pre-erythrocytic stage

Following the bite of the female mosquito, sporozoites circulate in the bloodstream for a very brief period. Some of the parasites invade hepatocytes, others being filtered out by a variety of non-specific mechanisms. At this stage the parasite would be susceptible to an antibody-mediated attack directed to components on the surface of the sporozoites. Such antibody could potentially exert its protective effect by any one of a variety of mechanisms, including opsonization, complement-mediated lysis or neutralization. Once invasion of hepatocytes has taken place HLA B-53 molecules presents processed antigen to the cytotoxic T cell. Then the parasites are killed by either direct lysis or by the range of soluble mediators (Marsh, 2002).

2.12.4 Erythrocytic stage

Once inside the red cells, the parasites appear well positioned to avoid host responses, but there are several gaps in its defenses. When parasites mature inside RBC it induces a series of morphological, functional antigenic changes in the host red cell membrane. Some changes are a result of alteration of host constituents, but others result from the parasite inserting its own molecules into the host cell membrane. Host immune system acts against these neoantigens. Cytokines produced by immune cells plays an important role against this stage (Marsh, 2002).

2.12.5 Natural killer cell

Natural killer (NK) cells derived from pluripotent hematopoietic stem cells are important cells of immune system that have two main functions: a cytolytic activity and a cytokine producing capacity. New insights into NK cell biology have suggested their major roles in the control of infections, particularly in *P. falciparum* infection (Mavoungoua, 2005). In a study Artavnis-Tsakonus and Riley have revealed that, in non immune donors NK cells are among the first cells in peripheral blood to produce IFN in response to *P. falciparum* infected red blood cells. The authors observed that NK cells are activated during the first 18 hours of exposure to parasitized erythrocytes. This activation was dependent on IL-12 and also to a lesser extent on IL-18. This observation suggests that NK cells may represent an early source of IFN gamma, a cytokine that has been implicated in induction of various antiparasitic effector mechanisms (Artavanis-Tsakonas and Riley, 2002).

It was also observed that during pregnancy cell function might be altered due to production of hormones and other pregnancy regulatory factors. A casual relationship between high cortisol levels and depressed NK cell cytotoxicity against *P. falciparum* parasitized erythrocytes and susceptibility to malaria has been demonstrated (Mavoungoua, 2005).

2.12.6 T cell

Both cellular and humoral arms of the adaptive immune system are pivotal elements in the eradication of plasmodium from the body, and both are critically dependent on CD4+ lymphocytes. Both the Th1 and Th2 subsets of CD4+ T cells have regulatory functions in the human malaria (Angulo and Fresno, 2002). CD8+ T cells have been implicated as critical effector cells in protection against pre erythrocytic stage malaria. It is established that role CD8+

T cells is absolutely dependent not only on IFN-ã and NO, but also on IL-12 and in part on NK cells (Doolan and Hoffman, 1999).

2.13 Laboratory Diagnosis of Malaria

2.13.1 Microscopic examination

Direct microscopic examination of intracellular parasites on both thick and thin Giemsa stained blood films is the current standard and the most widely practiced and useful method for definitive malaria diagnosis (Bloland, 2001). Advantageously providing; specie differentiation, quantification of the parasite density, and ability to distinguish clinically important asexual parasite stages from gametocytes it is critical for proper case-management and evaluating parasitological response to treatment, however slide collection, time-consuming staining/reading protocol; training and supervision of microscopist as well as skepticism associated with obtained results are limiting factors (Warhurst and William,1996*:* Bloland, 2001).

Anthony (2002) reported the development of certain fluorochromes which cause nuclear fluorescence in association with plasmodial nucleic acid by UV light at an appropriate excitation wavelength of 470 nm to 490 nm. The quantitative buffy coat (QBC) method as a modification of the light microscope has been adapted for malaria diagnosis (Hakim *et al*., 1993). By concentrating parasites at a predictable location after microhaematocrit tubes precoated with fluorescent Acridine Orange (AO) are centrifuged, the parasites can be viewed through the capillary tube using a special long-focal-length objective (paralens) with a fluorescence microscope **(**Craig and Sharp, 1997).

Although the QBC is more sensitive, requires less operational training and is less time consuming (Tharavanij, 1990). Electricity is always required, special equipment and supplies are

needed, the per-test cost is higher than simple light microscopy, and species diagnosis is non-specific (Bloland, 2001).

2.13.2 Rapid diagnostic tests (RDTS)

In view of the World Health Organization (WHO) recognition for the urgent need for new, simple, quick, accurate, and cost-effective diagnostic tests for determining the presence of malaria parasites and to overcome the deficiencies of light microscopy, numerous new malaria-diagnostic techniques were developed (Lowe *et al.*, 1996). According to Anthony (2002),these test target plasmodial protiens expressed on the red blood cell or soluble in blood, such as; *P. falciparum* Histidine Rich Protein (HRP) 1 and 2, a pan-malarial Aldolase and lactate dehydrogenase.

RDTs have been developed in different test formats with the dipstick and test strip been more satisfactory a device for safety and manipulation (Gaye *et al.,* 1996). RDTs detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies bound to a detectable marker; they do not require laboratory equipment (Tangpukdee *et al*., 2009). RDTs are convenient for utility and speed, however they fail to satisfy crucial diagonistic indices such as; detection threshold, quantification, specie differenciation and reliability of test result (Clinton *et al.,* 2009).

2.13.3 Serology

Serology detects anti-malarial antibodies against malaria parasites in serum, via either indirect immunofluorescence antibody (IFA) test or enzyme-linked immunosorbent assay (ELISA) (Center for Disease Control, 2015). Althoughserological markers are available for the four predominant species of human malaria, a positive test is of no clinical relevance to diagnosis of

acute infection as it only measures past experience, hence serology bridges the divide between diagnosis and treatment and is useful in blood screening prior transfusion (Maria *et al*., 2009).

2.13.4 Molecular tests

Detection of plasmodium genomic DNA usingpolymerase chain reaction techniques are becoming a more frequently used tool in the diagnosis of malaria, as well as the diagnosis of malaria and surveillance of drug resistance to chemotherapy (Bloland, 2001). According to Beck (1999),one important use of this technology is in detecting mixed infections or differentiating between infecting species when microscopic examination is inconclusive. With specific primers developed for each of the four species of human malaria, mixed infection detection and speciation is achievable when microscopic diagnosis is inconclusive (Beck, 1999). Although PCR cannot be considered a rapid technique for the initial diagnosis of malaria, its value lies in its heightened sensitivity, specificity, and speciation (Snounou *et al*., 1993; Dakic *et al*., 2014). Primary disadvantages to these methods are overall high cost, high degree of training required, need for special equipment, absolute requirement for electricity, and potential for cross-contamination between samples (Bloland, 2001).

2.13.5 Mass spectrometry

A novel approach for the *in-vitro* detection of the malaria parasite at a sensitivity of 10 parasites/μL of blood has been reported using direct ultraviolet laser desorption time-of-flight mass spectrometry (Mann, 2002). Quantification is rapidly achieved by intense ion signal associated with ferriprotoporphyrin IX (sequestered by malaria parasites during their growth in human red blood cells) which correlate with the sample parasitemia, hopefully, future improvements in the equipment and technique can make this method deployable and useful (Demirev *et al.*, 2002: Mann, 2002).

2.14 Malaria Treatment and Control

2.14.1 Economic burden and treatment of malaria

Malaria costs Africa more than $12 billion in loss of gross domestic product (GDP) every year and it is responsible for a 1.3 % growth penalty per year in some African countries, due to loss in productivity. It accounts for up to a third of all hospital admissions, and up to a quarter of all deaths of children under the age of 5 years. There are up to 800,000 infantile mortalities and a substantial number of miscarriages and very low birth weight (VLBW) babies a year due to the disease. The cost of malaria in economic terms is also high, treatment ranges in cost between $ 0.80 and $ 6.30 depending on local drug resistance, and the total cost in Africa is $ 1.8 billion per year. A bout of malaria typically costs 10 working days, adding to the economic burden.

The disease profile always has major economic consequences, although the full economic impact of epidemic malaria remains undefined. Specialized intervention approaches are needed for epidemic-prone areas, including enhanced surveillance activities and intensified anti-vector interventions. Such considerations are particularly critical at a time when malaria epidemics are occurring more frequently in Africa and throughout the World (WHO,2017).

2.14.2 Control and prevention of malaria

Soon after the Second World War, malaria was recognized by WHO not only as a health problem but also a socio-economic burden (Talisuna, 2004). In the late 1950s and early 1960s, the eradication of malaria seemed possible because the parasite does not have an animal reservoir and effective agents to interrupt transmission or to obtain a radical cure were available. On the basis of such observations, WHO spearheaded projects for malaria eradication by using indoor residual spraying and large mass drug administration programs using chloroquine (CQ) and pyrimethamine (Talisuna, 2004).Early in the Second World War, Dichloro Diphenyl

Trichloroethene (DDT) was developed and initially it was used to combat malaria. Expanded mosquito control program was carried out from 1955 to 1970**.** As a result malaria was eliminated from Europe and North America by judicious use of insecticides and manipulation of environmental and ecological characteristics. However, in many tropical and some temperate areas the disease is increasing. This is due to the wide spread of distribution of mosquito breeding place, large number of infected persons, inadequate resources, infrastructures and control programs (White, 2005).

2.14.3 Strategy for malaria control

The World Health Organization suggests that there are three essential elements of malaria control (DGHS and WHO, Bangladesh, 2006). First, is the selective vector control by reduction of the numbers of mosquitoe. Second, is early diagnosis, effective and prompt treatment of the cases. The third element is early detection or forecasting of epidemics and rapid application of controlmeasures **(**Phillips, 2001**).**

2.14.4 Prevention of malaria

Following measures can prevent malaria such as:

- Personal protection by mosquito nets, repellents, protecting clothing,
- Antimosquito measures.
- Prophylactic drugs.
- Vaccination against malaria.

2.14.4.1 *Personal protection*

Personal protection can be achieved by following measures: by using mosquito net, insecticide treated bed net, repellent creams and spray and by wearing of long sleeves and trousers (Bales and Gilles, 2002).

2.14.4.2 *Mosquito nets*

The use of mosquito nets as a protection from mosquito bites during the night has been practiced from very early times. However, if the nets are treated with an insecticide such as: permethrin, deltamethrin and lanbda eyhalothrin, they afford better individual and family protection against the mosquito (Bales and Gilles, 2002).

2.14.4.3 *Repellents*

These are substances applied to the skin, clothing or mosquito nets to repel mosquitoes. In the past citronella or eucalyptus oil was used on the skin. Several newer compounds are now available and among them N-diethyl-3-methyl benzamide (DEET) appears to be the best. It is available in wide range of concentrations and formulations such as: 50 percent solution in alcohol, lotions, creams, gels, aerosols and pump sprays. Mosquito coil or joss sticks containing pyrethrum are also used as repellent (Bale and Gilles, 2002).

2.14.4.4 *Protecting clothing*

Mosquito boots made of soft leather or canvas are useful to protect the ankles in the evening. Alternatively, a pair of thick socks may be pulled up outside the bottoms of trousers. Sleeves should be rolled down and trousers substituted for shorts or skirts after sunset (Beale and Gilles, 2002).

2.14.4.5 *Anti-mosquito measures*

These may be directed toward adult mosquitoes and their larvae. Destruction of adult mosquitoes can be carried out by spraying with insecticide, such as DDT or Gammexane. Anti-larval measures consists of elimination of breeding places of the mosquitoes and uses of larvicides (oil, Paris green, DDT dissolved in oil) and culture of Gappi fish, Telapya fish etc in small ponds (Beale and Gilles, 2002).

2.14.4.6 *Prophylactic drugs*

All travelers, unless eradication is complete, should take regular prophylactic drugs. Several, most of which are used for treatment of malaria, can be taken for prophylaxis. Generally, these drugs are taken daily or weekly, at a lower dose than curative dose (www.Wikipedia.com). For prophylaxis commonly used are drugs; mefloquine, doxycycline and Proguanil. The choice of drug is usually driven on the basis of the drug resistance status of the area (Jucket, 1999).

2.14.4.7 *Vaccination against malaria*

To immunize human against malaria, first attempt was taken in 1973 with irradiated sporozoites of *P. falciparum* and *P. vivax* (Gilles, 2002). Then several experimental malaria vaccines have been developed. They were so called sub-unit vaccines based on either the sporozoite or merozoite stage of the parasite (WHO, 1997). In between 1987- 1996, recombinant DNA vaccine, sporozoite vaccine, synthetic peptide vaccine and SPf66 vaccine were developed (Gilles, 2002). But yet, no successful vaccine for malaria is available as of June 2006. There are several reasons for the failure to develop an effective vaccine. These include: multi-stage life cycle, large genome, antigenic variation, complex immune response and immune evasion by parasites. Three main types of vaccine are currently under development:

-Pre-erythrocyte stage vaccine.

-Transmission-blocking vaccine.

-Asexual blood stage vaccine (Phillips**,** 2001).

2.14.4.8 *Roll back malaria*

Roll Back Malaria (RBM) is the title that has been given to a priority project of the World Health Organization (WHO). The movement was established on 23 July 1998 (Bales and Gilles, 2002).Roll back malaria is a global partnership of national governments, civil society, non-

governmental organizations, research institutions, professional associations, UN and development agencies, development banks, the private sector and the media. Roll Back Malaria aims to halve the world's malaria burden by 2010 (WHO, 2003).

The need for such an effort is abundantly clear: malaria places a huge burden on Sub-Saharan Africa, with 300 million people suffering acute illness each year, and one million dying, at least 70% of whom are children or pregnant women (Bales and Gilles, 2002). Roll Back Malaria was, therefore, called into being to promote an effective control strategy to combat the disease. This emphasizes rapid clinical case detection and treatment, use of insecticide treated bed nets, management of malaria during pregnancy, and focal control of malaria transmission in emergency or epidemic situations **(**Bales and Gilles, 2002).

2.15 Current Treatment of Malaria

Current drugs for the treatment of uncomplicated malaria are artemisinin based combination therapies (ACTs). This combination takes advantage of the rapid blood schizontocidal action of the artemisinin and the long duration action of the partner compound to affect rapid cure with low level of recrudescence. Severe malaria is a medical emergency and requires in-patient care. Deaths from severe malaria can result either from direct effect of the disease or the complications. It has been argued that with the limited number of antimalarial drugs available and the growing resistance of the parasites to these drugs, better responses to drug treatment and a significant slowing down of the rate of development of resistance can be achieved by combining antimalarial drugs (Frontline, 2005).

In January 2006, on the occasion of the release of the World Health Organization (WHO) guidelines for the treatment of malaria, WHO issued a press release urging 17 known companies

to stop marketing attempting monotherapies, and fore-direct their production efforts towards artemisinin-based combination therapy. The press release received major attention in the international media (newspapers, radio and television) and in the national press in endemic countries. At present, about 100 countries have adopted ACTs as recommended by WHO in the general health services. With increased mobilization of international funds, mainly from the Global Fund to fight AIDS, Tuberculosis and Malaria (GFATM), the procurement of ACTs for the public health sector has increased exponentially during the past few years (WHO, 2006).

2.16 Limitation of Current Treatment of Malaria

The drug treatment of malaria depends on the type and severity of the attack typically, guanine sulphate tablets are used and the normal adult dosage is 600mg every twelve hours, which can also be given by intravenous infusion if the illness is severe. *Plasmodium falciparum* malaria is a medical emergency that should be treated in the hospital. The type of drugs, the method of administering the drugs, and the length of the treatment depend on where the malaria was contracted and how sick the patient is. For all the strains (*P. vivax, P. ovale, P. malariae* etc) except *Falciparum*, the treatment for malaria is usually chloroquine and it's usually treated with a combination of quinine and tetracycline. Nowadays the ACTs are mostly in use. In countries where quinine resistance is developing, other treatments may include clindamycin (Cleocin) mefloquin (Lariam), sulfadoxine/pyrimethamine (Fansidar) or artesunate combination. Most patients receive an antibiotic for seven days. Those who are very ill may need intensive care and intravenous malaria treatment for the first three days. A patient with *Falciparum* malaria needs to be hospitalized and be given antimalarial drugs in different combinations and doses depending on the resistance of the strain.

The patient may need intravenous fluids, red blood cell transfusions, kidney dialysis, and assisted breathing. Drugs like primaquine or halofantrine may prevent relapses after recovery from *P. vivax* or *P. ovale*. These relapses are caused by a form of the parasites that remains in the liver and can reactivate months or years later. However, all these drugs maybe either toxic, ineffective or are not affordable to the common man, who is most affected by the disease (Philips, 2001).

2.17 Prophylaxis of Malaria

As there is no marketable vaccine available for protection against malaria despite decades of research, there is a need for an alternative method that offers a fairly reliable protection against malaria; since malaria can be severe in the non-immune, all visitors from a non-malarious area to a malarious area should be protected. Antimalarial drugs offer protection against clinical attacks of malaria.

The risk of contracting malaria depends on the region visited, the length of stay, time of visit, type of activity, protection against mosquito bites, compliance with chemoprophylaxis etc. pregnant women, infants and young children and people who have undergone splenectomy should avoid travel to a malarious area as these people are at higher risk of severe malaria. If travel is unavoidable, these people should take strict precautions to avoid mosquito bites and also take adequate chemoprophylaxis without failure (Kakkilaya, 2006).

Several drugs, most of which are also used for treatment of malaria, can be taken prophylactically. Generally, these drugs are taken daily or weekly, at a lower dose than would be used for treatment of a person who had actually contracted the disease. Use of prophylactic drugs is seldom practical for full-time residents of malaria-endemic areas, and their use is usually restricted to short-term visitors and travelers to malaria-endemic areas. Chloroquine and proguanil have an excellent safety record in the recommended dosages. Mefloquine 250 mg once

per week is not recommended for patients taken beta blocker or guanidine for pilots and others who need fine motor skills, known neurologic or psychiatric disorders, pregnant women in their first trimester or children less than 15 kg. Fansidar (S/P) has been associated with a relatively high incidence of potentially total reactions and is no longer recommended for prophylaxis but may be used for standby treatment. It should not be taken by pregnant women or by those who are sensitive to sulphonamides.

A serious adverse reaction includes toxic epidermal necrosis, agranulocytosis, hypersensitivity, pneumonitis and hepatitis. It has to be stressed here that no prophylaxis is full proof and failure arise most commonly from not taking the drugs as prescribed. In particular they must be started one week before departure and continued for 4-6 weeks after leaving malaria endemic areas. Any fever up to 12-18 months after leaving a malaria endemic should arouse suspicion of malaria and be investigated accordingly, appropriate advice must be sought prior to departure from a reputable travel advice center (Wyler, 1993).

2.18 Anti-malarial Drug Resistance

Antimalarial drug resistance is the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject. The drug in question must gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action (Bruce-Chwatt, 1982). This definition of resistance requires demonstration of malaria parasitaemia in a patient who has received an observed treatment dose of an antimalarial and simultaneous demonstration of adequate blood drug and metabolite concentrations using established laboratory methods (Plowe, 1995; Su, 1997; White, 1997) a distinction must be made

between a failure to clear malarial parasitaemia or resolve clinical disease following a treatment with an antimalarial drug and true antimalarial drug resistance, while drug resistance can cause treatment failure, not all treatment failure is due to drug resistance.

Many factors can contribute to treatment failure including incorrect dosing, non-compliance with duration of dosing regimen, poor drug quality, drug interactions, poor or erratic absorption, and misdiagnosis. Probably all of these factors, while causing treatment failure or (apparent treatment failure) in the individual, may also contribute to the development and intensification of true drug resistance through increasing the likelihood of exposure of parasites to a suboptimal drug levels.

2.18.1 Mechanisms of anti-malarial resistance

In general, resistance appears to occur through spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs. For some drugs, only a single point mutation is required to confer resistance while for others, multiple mutations appear to be required provided the mutations are not deleterious to the survival or reproduction of the parasite, drug pressure will remove susceptible parasites while resistant parasites survive. In the case of malaria, a single malaria isolates have been found to be made up of heterogeneous populations of parasites that can have widely varying drug response characteristics, from highly resistant to completely sensitive. Similarly, within a geographical area, malaria infections demonstrate a range of drug susceptibility. Over time, resistance becomes established in the population and can be very stable; persisting long after specific drug pressure is removed (Thaithong, 1983). For example, the biochemical mechanism of resistance of chloroquine is that, when the malaria parasite digests hemoglobin, large amounts of toxic by-products are formed. The parasite polymerizes this by-product in its food vacuole, producing, non-toxic haemozoin (malaria pigment). It is believed that resistance of *P. falciparum* to chloroquine is related to an increased capacity for the parasite

to expel chloroquine at a rate that does not allow chloroquine to reach levels required for inhibition of haem polymerization. This chloroquine efflux occurs at a rate of 40 to 50 times faster among resistant parasites than sensitive ones. Further evidence supporting this mechanism is provided by the fact that chloroquine resistance can be reversed by drugs which interfere with the efflux system. It is unclear whether parasite resistance to other quinolone antimalarials (amodiaquine, mefloquine, halofantrine and quinine) occurs via similar mechanisms (Foley and Tilley, 1997).

2.19 Current Trends in Anti-Malarial Therapy

2.19.1 Combination therapy

According to Naser (2007) the current practice in anti-malarial chemotherapy is multifaceted. Offering several advantages, the first line of action in combating malaria, is the optimization of therapy by combining two or more existing anti-malarial agent (Philip, 2003). Combination therapy in common use, include; artemisinin based combination therapy, artovaquone/proguanil, amodaquine/sulfadoxine/pyrimethamine and chlorproguanil/dapsone (Naser, 2007). Muheki *et al*. (2004) reported that the use of artemether-lumefantrine (Coartem) in an area of resistance to pyrimethamine-sulfadoxine resulted in very high cure rates (99%) and reduced gametocyte carriage 2 years after, malaria cases fell by 94% and costs by 88% due to the combined effects of the introduction of effective treatment as well as control measures.

The forgoing result was in consonance with earlier studies on artesunate-mefloquine combination therapy by Nosten *et al.* (2000). According to Kublin *et al*. (2000) another intriguing possibility is the reuse of chloroquine, ideally in combination regimens, in areas where it has not been used for an extended period. In view of the forgoing, Pfizer developed a fixed dose combination of Azithromycin (250mg) and Chloroquine (150mg) for prophylactic use

during pregnancy. Data from phase III clinical trial indicates synergy and high efficacy in areas where chloroquine resistance is high (Ollioro *et al*., 2009). Phillip (2003) reiterated that the key advantage of chlorproguanil/dapsone is a relatively short half-life, long enough to provide effective therapy with 3-day daily dosing but short enough to readily select for resistance. He also suggested that its optimal use may be with the rapid potency of artesunate but definitive studies of this combination are still needed (Philip, 2003).

2.19.2 Chemical modification of existing anti-malarial agents

Improvement upon existing anti-malarials by chemical modifications of these compounds is another approach to improving chemotherapeutic outcome. This strategy birthed novel anti-malarials. For example, chloroquine, primaquine and mefloquine were discovered through chemical strategies to improve upon quinine (Stocks *et al.,* 2001) and 4-aminoquinolines that are closely related to chloroquine appear to offer the antimalarial potency of the parent drug, even against chloroquine-resistant parasites (Kaschula *et al.,* 2002).

An 8-aminoquinoline, tafenoquine, offers improved activity against hepatic-stage parasites over that of the parent compound primaquine (Walsh *et al.,* 1999), and is effective for anti-malarial chemoprophylaxis (Naser, 2007). The analog lumefantrine was developed from halofantrine due to its toxicity and is now a component of the new combination co-artemether (VanVugt *et al.,* 2000). Better tolerated in G6PD deficiency and a propensity for anti-relapse activity is Bulaquine a congener of primaquine (Naser, 2007). New folate antagonists (Tarnchompoo *et al.,* 2002) and new endoperoxides related to artemisinin (Vennerstrom *et al.,* 2000; Posner *et al.,* 2003) are also under study.

2.19.3 Exploration of ethno-based products

Currently, Plant-derived natural products are the sources of the two most important drugs currently available to treat severe falciparum malaria and folkloric knowledge/use of medicinal plants among natives from malarious regions continues to provide ethnomedical data (Shanker *et al.,* 2012). From *in-vitro* and *in-vivo* studies, Uchoa *et al*. (2010) observed a high therapeutic index and confirmed the anti-malarial activity of fractions; chiefly beta- sitosterol and tormentic acid from *Cecropia pachystachya* (Cecropiaceae) a plant species largely used to treat fever (including that caused by malaria parasites) and as food by native indigenous populations in the Amazon Region of Latin America.

An *in-vtro* and *in-vivo* assessment of the essential oils obtained from *Vanillosmopsisarborea*, *Lippiasidoides* and*Crotonzehntneri*, revealed robust representation of Monoterpenes and Sesquiterpenes in the fractions. Individual essential oil constituents includes α-bisabolol, estragole, and thymol, which exhibited good activity against *P.falciparum* as well as low toxicity (Mota *et al.,* 2012). Botte*etal*., (2012) reported that Molecules such as polyketide cycloperoxides isolated from the marine sponge, *Plakortis simplex* and xesto-quinonefrom the sponge, *Xestospongia* sp., have antiplasmodial activity.

2.19.4 Anti-malarial evaluation of compounds active against other diseases

Although Folate antagonists, tetracyclines and other antibiotics were developed for their antibacterial properties, they were later found to be active against malaria parasites (Clough and Wilson, 2001). Atovaquone development was expedited by the discovery of its activity against *Pneumocystis,* its potential as an anti-malarial in combination with proguanil was re-explored, and found to have marked anti-malarial synergy (Canfield *et al.,* 1995). It is thus expedient to screen new antimicrobial agents and other available compounds for anti-malarial activity, as they

may provide a relatively inexpensive means of identifying new anti-malarials. (Gelb *et al.*, 2003).

2.19.5 Anti-sequestration compounds

Inhibition of cell adhesion processes is becoming increasingly interesting in the discovery of novel therapeutics (Simmons, 2005). An interesting possibility lies in compounds which prevents the parasite from causing severe forms of the disease rather than killing, by way of hampering the parasite's ability to perform cytoadhesion as mediated by adhesins (Naser, 2007; Krintratun *et al.,* 2007). Sequestration in brain and placenta play an important role in the pathogenesis of cerebral and placental malaria, respectively (Rowe *et al.,* 2009*:* Rogerson *et al.,* 2010). Efforts at inhibiting cytoadherence mediated by plasmodia receptors have been rewarded. CD36-mediated cytoadherence can be inhibited *in vitro* by antiretroviral such as; ritonavir and saquinavir (Nathoo *et al.,* 2003), and polysaccharides (carrageenans) derived from seaweed (Andrew *et al.,* 2005; Adams *et al.,* 2007). Using the crystal structure of intracellularadhesion molecule 1 (ICAM1), (+)-epigalloylcatechin gallate, a naturally occurring polyphenol compound in green tea, inhibited cytoadherence to ICAM1 in a dose-dependent manner (Dormeyer *et al.,*2006).

Although detailed molecular mechanisms of parasite adhesion is still unclear, the foregoing findings open up the possibility of developing therapeutic interventions aimed at blocking or reversing parasite adhesion (Brain and Rajeev, 2011).

2.19.6 Exploring drug resistance reversers

Although chloroquine at first-line anti-malarial in most of the world appears to already have failed, this inexpensive, rapid acting, well-tolerated anti-malarial may be resurrected by its combination with effective resistance reversers (Sowunmi *et al*., 1997).Although unacceptably high concentrations of the resistance reversers are needed for their effects in many cases,

however Van and colleagues demonstrated that combinations of two or more of these agents (verapamil, desipramine and trifluoperazine) at non-toxic concentrations may provide clinically relevant resistance reversal, hence restore chloroquine efficacy (Van Schalkwyk *et al.,* 2001).

Tri-cyclic Acridones possessing a short alkyl amine chain attached to the central nitrogen atom, in addition to its anti-malarial propensity, could make chloroquine resistant parasites susceptible to the drug again by means of blocking the PfCRT Pump protein (Kelly *et al.,* 2009).

2.19.7 Discovery of compounds active against new drug targets

In contrast to the cytosol, the Apicoplast of plasmodium species has a prokaryote-like genome and includes a number of biochemical pathways that are present in bacteria, plants and apicomplexan parasites but are absent in the human host and thus provide obvious opportunities for chemotherapy (Istvan et *al*., 2010). Globin hydrolysis mediated by a number of classes of proteases appears to offer potential targets for chemotherapy (Jiang *et al*., 2001;Nezami*et al*., 2002: Noteberg *et al*., 2003). Falcipain inhibitors have been shown to prevent hemoglobin hydrolysis with the accumulation of intact hemoglobin in the food vacuole, inhibition of parasite development and curative activity (Batra *et al*., 2003; Shenai *et al*., 2003). Razakantoanina *et al*. (2000) demonstrated the *in-vitro*antimalarial activity of some *plasmodial* lactate dehydrogenase (pLDH). In view of developmental demands, host dependent Synthesis of phosphatidylcholine (the most abundant lipid in plasmodial membranes) is one obvious target with great potential. Blockage of choline transport has been identified as a promising therapeutic strategy (Vial and Calas, 2001). From independent studies carried out by Calas *et al*. (2000) and Wengelnik *et* al. (2002), the lead compound-G25, inhibited the development of cultured *P. falciparum* parasites displaying an outstanding *in-vivo* therapeutic index.

Transport pathways unique to malaria parasites also constitute membrane targets. Although parasite transport mechanisms is not well understood (Kirk, 2001).Exploitingthe selective transport of cytotoxic compounds into *P. falciparum*-infected erythrocytes was reported by Gero *et al*. (2003) using dinucleoside phosphate dimers conjugated to anti-malarial compounds to improve selective access to parasite targets.Using thiolactomycin, Waller *et al*. (1998) in an earlier study exploited the type II fatty acid biosynthesis pathway (β-ketoacyl-acyl-carrier protein-synthase-FabH) and showed that this antibiotic was active against cultured malaria parasites.

2.19.8 Malaria vaccine development

With the growing trend of both insecticide and anti-malarial drug resistance, the development of a malaria vaccine carries herculean expectations, about 6000-8000 malaria proteins have so far been identified, however few have been the subject of clinical trials (Philippe *et al.,* 2002). Vaccines can target different stages of the parasite's cycle offering protective immunity (Philippe *et al.,* 2002).

Though reports from experimental means of achievement have so far failed (Brown *et al.,* 2002), moreover immunization with sporozoites was reported to confer protective immunity (Riley *et al.,* 2013). In view of the forgoing, pivotal challenges include; substantial polymorphism in immunologically important regions of the proteins and low immunogenicity. Thus emphasis is being placed on molecules derived from the latter stages (Druilhe *et al.,* 1998).While asexual blood-stage vaccines aim at reproducing antibody-mediated protection acquired through repeated exposure to infection (Philippe *et al.,* 2002). Gamete-stage vaccine aims to prevent mosquitoes that are feeding on an infected individual from acquiring and transmitting the parasite. This

altruistic approach however does not confer protection on the vaccinated individual but contributes to protection in the community (Carter *et al.,* 2000).

A completely effective vaccine is not yet available for malaria; the SPf66 developed by Pattarroyo did not show any efficacy. Other vaccine candidates, targeting the blood-stage of the parasite's life cycle such as; MSP1-3, AMA1, EBA 175 and P*f*s25 have also been insufficient on their own (Graves and Gelband, 2006). The engineering of RTSS (Mosquirix) by Glaxo Smith Kline induced high antibody titers that block the parasite from infecting the live it is currently the most developed vaccine (Malaria vaccine initiative PATH, 2013). It provided protective immunity to 7 out of 8 volunteers challenged with *P. falciparum* as well as against both clinical and severe malaria in young infants (Malaria vaccine initiative PATH, 2013).

In view of the failures with initial available candidates, recent trends gravitate towards multistage vaccines that use combinations of components that are individually not sufficiently effective, hence the use of mixtures of five or more antigens. However this combo strategy must surmount; immunogenic, financial, safety, technical and analytical challenges (Philippe *et al.,* 2002).In view of the foregoing, the malaria vaccine advisory committee to the world health organization outlined a "Malaria Vaccine Technology Roadmap" that has as one of its landmark objectives: ―to develop and license a first-generation malaria vaccine that has a protective efficacy of more than 50% and lasts longer than one year" by 2015 (Hoffman *et al*., 2002).

2.19.9 Plasmodial biochemistry

Elucidation of novel plasmodial biochemical pathways continues to open up drug targetscrucial to plasmodial metabolism (Gero *et al*., 2003; Shenai *et al*., 2003). The crucial role Aminoacyl-tRNA synthetases play in protein translation, Pham *et al*. (2014) acknowledged the considerable

shift in research interest from bacteria and fungi aminoacyl-tRNA synthetases to that found in eukaryotic parasites. Crystal structures have now been solved for many parasite tRNA synthetases, and opportunities for selective inhibition are becoming apparent. Istvan *etal*. (2011) showed that the *Plasmodium* cytosolic IleRS was inhibited by the isoleucine analogue-Thiaisoleucine whose rapid *ex-vivo* antimalarial activity was largely attributed to competition with isoleucine for binding to the synthetase prior to its incorporation into newly translated proteins.

According to Shibata *et al.* (2011) few differences exist in active site residues between parasite and host. An *in-vitro* study by Istvan *et al*. (2011) revealedthat *P.falciparum's* cytoplasmic IleRS (PF13-0179) is 47.5% identical to human IRS, whereas its apicoplast IRS (PFL1210w) is more similar to bacterial IRS. Although Thiaisoleucine was not considered a potential drug lead due to its limited potency against parasites and possibly low therapeutic index, it was regarded a valuable tool for studying Isoleucine acquisition and show that simple isoleucine analogs can be incorporated by cytosolic IRS resulting in parasite toxicity. A potential source of concern is the development of bacterial resistance and treatment failure reported for this drug target (Ranade *et al*., 2013). According to Holm *et al*. (1996), approximately one-third of proteins are metalloproteins, which serve to execute a wide array of functions in vivo, including regulating blood pH, facilitating matrix degradation, modulating DNA transcription, and many others. Given the importance of these functions, metalloenzyme misregulation plays a significant role in human disease, and its inhibition offers an appealing approach to disease treatment (Day and Cohen, 2013). Typically, metalloenzyme inhibitors especially hydroxamic acids, are drug-like small molecules that incorporate a metal binding group (i.e the hydroxamate moeity) in order to coordinate the active site metal ion, leading to enzyme inactivation (Day and Cohen, 2013). The

zinc metalloenzymes; M1 alanine aminopeptidase (PfM1AAP) and M17 leucine aminopeptidase (PfM17LAP) are believed to play a role in the terminal stages of digestion of host hemoglobin, a generating a pool of free amino acids that are essential for parasite growth and development (Skinner-adams *et al*., 2012).

Furthermore, Skinner-adams *et al*. (2012) reported that the experimental hydroxamate based compound-CHR-2863 is a potent inhibitor(*Ki* = 2.4 μM) of malaria aminopeptidases,binding strongly and chelating the active site's metal ion via the terminal hydroxamic acid region.

Similarly, Umeda *et al*. (2011) reported thatFosmidomycin ahydroxamate basedcompound proven to be efficient in the treatment of *P. falciparum* malaria by inhibiting 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), an enzyme of the non-mevalonate pathway, absent in humans. Crystal structures of fosmidomycin-bound complete quaternary complexes of *plasmodium falciparum* DXR revealed that *cis* arrangement of the oxygen atoms of the hydroxamate group of the bound inhibitor is essential for tight binding of the inhibitor to the active site metal.Indiscriminate metalloenzyme inhibition or metal ion disregulation by metal ion removal from non-target metalloproteins, especially by structurally simple hydroxamate based compoundscreats the perception of a greater risk in obtaining clinically successful therapeutics (Day and Cohen, 2012).

2.20 Intracellular Sites of Action of Antimalarial

Antimalarials have been investigated to be active during the stage when the parasite is located inside the RBC, in other words, the drug regimens target mainly the erythrocytic cycle of the *Plasmodium* life cycle (Ridley, 2002). During the blood stage of the *Plasmodium* parasites between 60-80 % of the haemoglobin is broken into haem (Egan, 2008). The latter is said to be harmful to the parasites. The parasites, however, neutralize the haem during the process of

polymerization of haemazoin. This polymerization is inhibited by antimalarials such as chloroquine mefloquine, halofantrine, lumefantrine (Hempelmann, 2007) and artemisinins; resulting in the accumulation of haem and eventual death of the parasites.

Antimalarial drugs, therefore, act by preventing globin lysis and haemazoin formation. Quinoline antimalarials such as chloroquine, for example, act by inhibiting the synthesis of the hemozoin pigment when they get trapped in the digestive vacuole of the parasite due to the extra proton they gain due to the acidic environment of the vacuole (Jiang *et al*., 2006). The malaria parasites use hemoglobin/ hemazoin to make amino acids which are vital for their existence. According to Gavigan *et al*. (2001), hemoglobin hydrolysis by the *Plasmodium* parasite (trophozoite) is mediated by a number of classes of enzymes (proteases) in the food vacuole including 49 aspartic and cysteine proteases and falcilysin.

These proteases appear to mediate the invasion and rupture of infected erythrocytes as well, possibly to get through the erythrocyte cytoskeleton, a complex network of proteins. In addition, a number of malarial proteins are made during the late schizont and merozoite life-cycle stages and likely to aid in the complex series of events involved in the RBCs (Hall *et al.*, 2005). Although the exact roles of various classes of proteases are not entirely clear, inhibitors of cysteine and serine proteases have consistently repressed erythrocyte rupture and invasion. Studies have found that the biological activity caused by drugs results in the swelling of the parasite, as well as the inhibition of the proteolytic activity of these enzymes (Semenove *et al*, 1998).

Malaria parasites are said to be sensitive to the free radicals produced by some antimalarial drugs including artemisinin-related compounds. Artemisinin, in the presence of haem inhibits the

polymerization of haemazoin by alkylation (Mishra *et al*., 2009). The haem or Fe^{2+} cleaves the peroxide bridge in the artemisinin compound leading to the formation of free radicals (Ridley, 2002). These radicals then alkylate one of the pyrazole rings in the haem nucleus, hence inhibiting the formation of haemazoin. Other modes of action shown by antimalarials include pyrimidine synthesis inhibition. Inhibiting this synthesis would mean inhibiting the growth of the parasites (Rosenthal, 2003). An example of such an antimalarial drug is atovaquone which inhibits the respiratory chain of malaria mitochondria at complex III (Ridley, 2002). Some folate antimalarials such as pyrimethamine and sulfonamides inhibit the formation/ synthesis of dihydrofolate, which is necessary in the biosynthesis of pyrimidines. Iron chelators, on the other hand, are active by preventing iron from entering important metabolic pathways of parasites within the RBCs, as well as combining with iron, forming toxins to the parasites. Among potential targets for new modes of chemotherapy are malarial proteases. Cysteine and aspartic protease inhibitors are now under study as potential antimalarials. The increased understanding of the mode of action of a compound will contribute in the mitigation in the rapid spread of resistance against presently used antimalarials as some antimalarials given in combination treatments may have counter effects, causing a relapses of the parasite load (Gavigan *et al*., 2001).

2.21 Traditional Medicine

Traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness, while a medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs. Humans have used them throughout history to

either cure or lessen symptoms from an illness. A pharmaceutical drug is a drug that is produced in a laboratory to cure or help an illness (WHO, 2002).

The world have a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value (Sandhu and Heinrich, 2005; Gupta, 2011). Human beings have used plants for the treatment of diverse ailments for thousands of years (Junaid *et al.,* 2006; Sofowara, 2008). According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rabe and Van Stoden, 2000), since they cannot afford the products of Western pharmaceutical industries (Salie *et al.,* 1996), together with their side effects and non-availability of healthcare facilities (Griggs *et al*., 2001). Rural areas of many developing countries still rely on traditional medicine for their primary health care needs. These medicines are relatively safer and cheaper than synthetic or modern medicine (Ammara *et al.*, 2009). People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines (Van Wyk *et al.,* 2000). Herbal medicines are in great demand in both developed and developing countries as sources of primary health care owing to their wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are reportedly safe and promises to overcome the resistance dilemma developed by the pathogens as such herbal mixtures exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Tapsell *et al.,* 2006).

Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people. Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources (Fabricant and Farnsworth, 2001). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Mahesh and Sathish, 2008).

2.22 Traditional Knowledge of Malaria and Its Treatment

Medicinal plants remain a major source of drugs in the treatment of various categories of human ailments especially in the developing countries. They have formed the basis for traditional medicine systems, which have been used for thousands of years in African countries. The World Health Organization estimates that 80% of the world's inhabitants continue to rely mainly on traditional medicine systems for their health care. Herbal traditional medicine has the potential to improve the health of developing countries and contributes immensely to strategic reduction of excess mortality, disability and other risk factors to human health (WHO, 2002). In the modern world, interest in the therapeutic value of medicinal plants are getting revived and attention is being directed to explore and evaluate the efficacy of herbal drugs for the treatment of various diseases, including malaria, as also for those which do not respond adequately to synthesized drugs.

The new interest in the strategies on malaria treatment and control is to investigate the folkloric medicine in the search for potent antimalarials, since approximately 80 percent of the affected populations still depend on traditional medicine as its primary source of treatment of the diseases (WHO, 2002). For example artemisinin isolated from the herb *Artemisia annua,* which has been

in use in traditional Chinese medicine as a remedy for chills and fever for more than 2000 years. Clinical studies have shown the drug to be a safe and effective antimalarial agent even in the case of chloroquine-resistant *Plasmodium falciparum* malaria including those of cerebral malaria.

2.22.1 Traditional knowledge of malaria and its treatment in Nigeria

It is a known fact that the oldest component of the Nigeria health sector consists of traditional healers and birth attendants. They are the providers of primary healthcare. These healers provide a client -centered and personalized health care that is culturally appropriate, holistic and tailored to meet the needs and expectations of the patients. In Northern Nigeria where malaria is endemic, there has been a traditional use of plants as antimalarials, although without proven scientific justification. The relevant ethno-botanical, pharmacological and toxicological studies may not be available (Omulokoli *et al.*, 1997).

Traditional healers across the northern region of Nigeria claim that traditional knowledge of malaria and their remedies offer a huge potential to fight the disease. Even though there is no sufficient scientific evidence, and there is also lack of evidence of quality control measures, safety, dosing and toxicity of the remedies, the healers continue to enjoy the confidence of the local people who continue to patronize them. The herbal remedies used by the healers for the treatment of malaria are also mostly in the form of decoctions or infusions of leaves, stem-barks, roots and other parts of the plants. The traditional healers claim that they find many plant species to be effective against malaria in their daily practices, and investigations have revealed that many of such plants are being selected and screened for their antimalarial properties in-vivo and /or in -vitro (Munoz *et al.*, 2000). Traditional medicines have been used to treat symptomatic malaria for hundreds of years (Willcox *et al.*, 2004). These medicinal herbs are still used today by the

majority of the rural populations in developing countries (Mohammed, 2009). A need for traditional medicine as a source of malaria treatment has been recognized in view of the difficulties faced in areas where populations are either unable to afford or access effective antimalarial or are unwilling to use allopathic medicines (Van, 2001). Artemisia annual plant has been used as a traditional remedy for chills and fevers for more than 2000 years by the Chinese. Artemether, a derivative from artemisinin, is found to be more active than its precursor and is the most frequently used artemisinin derivative as a first line treatment for uncomplicated malaria (WHO, 2008).

To assure the development of efficient and safe malaria phytomedicines, the use of these medicinal plants needs to be scientifically studied (i.e. critiqued and standardized). The pharmacological efficacy, phytochemical composition, as well as the toxicity of these plants needs to be investigated as potential antimalarial medicines.

2.23 Natural Properties of Plant Secondary Metabolites

The plant chemicals are classified as primary or secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants such compounds are often concentrated in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism. Primary metabolites obtained from higher plants for commercial use are high volume-low value bulk chemicals (e.g. vegetable oils, fatty acids, carbohydrates etc.) (Achten *et al*., 2010).

Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute important sources of antiparasitic infection, pesticides and many pharmaceutical drugs. For a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing important roles in the human society to

combat diseases (Wink *et al.,* 2005). Secondary metabolites (compounds) have no apparent function in a plant‘s primary metabolism, but often have an ecological role, as pollinator attractants, represent chemical adaptations to environmental stresses or serve as chemical defense against micro-organisms, insects and higher predators and even other plants (allelochemics). Secondary metabolites are frequently accumulated by plants in smaller quantities than the primary metabolites (Sathishkumar and Paulsamy, 2009).

In contrast to primary metabolites, they are synthesized in specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result, secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products than the primary metabolites (e.g. steroids, quinines, alkaloids, terpenoids and flavonoids), which are used in drug manufacture by the pharmaceutical industries. These are generally obtained from plant materials by steam distillation or by extraction with organic or aqueous solvents and the molecular weight are generally less than 2000. Some biologically active plant compounds have found application as drug entities or as model compounds for drug synthesis and semi-synthesis (Paulsamy, 2009). A survey of current pharmaceutical products for medical uses revealed that, of the total prescription drugs dispensed, 25% are plant derived (Ogundipe *et al.,* 1998).

Plant compounds are highly varied in structure. Many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives. However, there have been increased attention on extracts and biologically active compounds isolated from plant species used in herbal medicine, due to the side effects and the resistance that pathogenic micro organisms build against the synthetic antibiotics (Essawi and Srour, 2000). New compounds inhibiting microorganisms such as benzoin and emetine have been isolated from plants. Of the various pharmaceuticals used in

modern medicine, aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine serve as examples of drugs discovered through observations of traditional medical practices (Gilani and Rahman, 2005). Eloff (1999) stated that the antimicrobial compounds from plants may inhibit bacteria by different mechanisms than the presently used antibiotics and may have clinical value in the treatment of resistant microbial strains.

Plant constituents may be extracted and used directly as therapeutic agents or as starting materials for drug synthesis or as models for pharmacologically active compounds in drug synthesis. The general research methods include proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemo pharmacological investigations, toxicological and clinical studies, standardization and use of active moiety as the lead molecule for drug design (Wink *et al.,* 2005).

2.24 Plant Secondary Metabolite

2.24.1 Terpenoids

The essential oils of plants are secondary metabolites that are highly enriched with compounds known as terpenes. These compounds are based on an isoprene structure with the general formula of $C_{10}H_{16}$ (Cowan, 1999). Terpenes and terpenoids constitute a very large family of compounds. The structures of terpenoids are diverse and range from relatively simple linear hydrocarbon chains to highly complex ring structures (Back and Chappell, 1996). Terpene hydrocarbons may occur as monoterpenes (C_{10}), diterpenes (C_{20}), triterpenes (C_{30}), tetraterpenes (C_{40}), hemiterpenes (C_5) and sesquiterpenes (C_{15}). Terpenes that contain an additional element (usually oxygen) are termed terpenoids (Cowan, 1999). Triterpenes have been found to be strong inhibitors of HIV-1 reverse transcriptase *in vitro* (Bessong *et al*., 2004).

2.24.2 Alkaloids

Alkaloids are organic bases containing nitrogen in a heterocyclic ring. Many have pronounced pharmacological activity (Williamson *et al*., 1996). The first medically useful example of an alkaloid is morphine which was extracted from opium poppy *Papaver somniferum* in 1805 (Fessenden and Fessenden, 1982).

Some alkaloids have both antiplasmodial and antimicrobial properties (Karou *et al.*, 2006), while others may be useful against HIV infection as well as intestinal infections associated with AIDS (McMahon *et al*., 1995). The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine is attributed to their ability to intercalate with DNA (Cowan, 1999) while indoloquinoline alkaloids such as cryptolepine, cause cell lysis and morphological changes of *Staphylococcus aureus* (Sawyer *et al*., 2013). Berberine is found in roots, rhizomes and stem bark of plants. Extracts and decoctions containing berberine have significant antimicrobial activity against organisms such as bacteria, viruses, fungi, protozoans, helminths and Chlamydia (Karou *et al.,*2006). Clinically, berberine is used in the treatment of bacterial diarrhoea due to its ability to reduce intestinal secretion of water and electrolytes, induced by cholera toxin, as well as inhibition of some *Vibrio cholerae* and *Escherichia coli* enterotoxins (Karou *et al.,* 2006).

2.24.3 Phenolics and polyphenols

Phenolic compounds include a wide range of secondary metabolites found in plants. They possess in common an aromatic ring substituted by one or more hydroxyl groups (Harborne, 1994). The main classes are simple phenols, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids (flavanols, flavones, flavanones, isoflavones and anthocyanins), chalcones, aurones, hydroxycoumarins, lignans, hydroxystilbenes and polyflavans (Krueger *et al*., 2003). The

common representatives of a wide group of phenylpropane-derived compounds that are in the highest oxidation state are cinnamic and caffeic acids. Caffeic acid, which is effective against viruses, bacteria, and fungi, is found in common herbs such as tarragon and thyme (Cowan, 1999).

Catechin and pyrogallol are both hydroxylated phenols, shown to be toxic to microorganisms. The mechanisms thought to be responsible for phenolic toxicity microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with the proteins (Alghazeer*et al.*,2012). Eugenol is a well-characterised representative found in clove oil. Phenolic constituents present in essential oils are generally recognized as active antimicrobial compounds. Eugenol, carvacrol, and thymol are phenolic compounds in cinnamon, cloves, sage, and oregano that possess antimicrobial activity. The exact causeeffect relation for the mode of action of phenolic compounds has not so far been determined. However, researches indicated that they may inactivate essential enzymes, reacting with the cell membrane or disturbing material functionality (Eruteya and Odunfa, 2009).

2.24.4 Tannins

Tannin is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatine from solution, a property known as astringency (Haslam, 1996). Tannins are found in almost every plant part bark, wood, leaves, fruits and roots. They are divided into two groups namely hydrolysable tannins which are based on gallic acid or ellagic acid, and usually occur as multiple esters with D-glucose; and condensed tannins which are derived from flavonoid monomers. Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins among others.

Both hydrolysable and condensed tannins have been found to be strong inhibitors of HIV-1 reverse transcriptase *in vitro* (Bessong *et al*., 2004).

2.24.5. Flavonoids: flavones and flavonols

Flavonoids are an important group of polyphenols, widely distributed in plant flora. About 4000 flavonoids are known to exist and some of them are pigments in higher plants. Flavones are phenolic structures containing one carbonyl group. The addition of a 3-hydroxyl group yields a flavonol (Gandhi *et al.*,2009). The common flavonoids found in plants are quercetin and kaempferol. Flavonoids are derived from parent compounds known as flavans. Since they are known to be synthesised by plants in response to microbial infection, they have been found to be effective antimicrobial and anti parasitic substances against a wide array of infections. Their activity may be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Gandhi raja *et al.,* 2009).

2.24.6 Saponins

Saponins are a vast group of glycosides, widely distributed in higher plants. They are abundant in many foods consumed by animals and man (Soetan *et al.,* 2006). Saponins are distinguished from other glycosides by their activity in decreasing surface tension. Many saponins have pharmacological properties and are used in phytotherapy and in the cosmetic industry (Sparg *et al*., 2004). Saponins can be classified into two groups based on the nature of their aglycone skeleton. Steroidal saponins are almost exclusively present in the monocotyledonous angiosperms, while triterpenoid saponins occur mainly in the dicotyledonous angiosperms (Soetan *et al.,* 2006). Saponins are believed to form the main constituents of many plant drugs and folk medicine, and are considered responsible for numerous pharmacological properties (e.g. ginseng constituents). The two acylated bisglycoside saponins, Acaciaside A and B, isolated

from *Acacia auriculiformis* have been demonstrated to posses antifungal and antibacterial activity (Mandal *et al.*, 2005).

2.24.7 Quinones

Quinones may be defined as aromatic rings with two ketone substitutions. Quinones are characteristically highly reactive. They are responsible for the browning reaction in cut or injured fruits and vegetables which happens because of polymerisation in the presence of oxygen and are an intermediate in the melanin synthesis pathway in human skin (Gokhale and Wadhwani, 2015). Oxidation and reduction reactions allow an easy switch between diphenol and diketone.

Vitamin K is a complex naphthoquinone that possesses antihaemorrhagic activity which may be related to its ease of oxidation in body tissues (Gokhale and Wadhwani, 2015). Quinones may complex irreversibly with nucleophilic amino acids in proteins (Gokhale and Wadhwani, 2015), which leads to inactivation of the protein and loss of function.

2.24.8 Sterols

Plant sterols are C28 and C29 carbon steroid alcohols 12 that are integral components of plant cell membranes, have been shown to be key components of plant plasma membrane microdomains and may exert similar functions in human cells. These compounds cannot be synthesized by humans and are introduced through the diet where they are found concentrated in plant foods,especially those with are lipid rich.

2.25 Jatropha curcas

Jatropha curcas L. (Euphorbiaceae) commonly known as physic or purging nut is an all-purpose, zero-waste perennial plant (Datta, 2007). This plant natively occurs in India, Africa and North America. The genus name *Jatropha* is derived from the Greek *iatrós* (doctor) and *trophé* (food) which implies medicinal use.Natural products isolated from *Jatropha curcas* are found to be the

excellent source of synthetic and traditional herbal medicine. The plant survives with minimum inputs in many parts of the country. *Jatropha*, the wonder plant, produces seed with an oil content of 37%. *Jatropha curcas* is gaining importance commercially as a green fuel source and is being advocated for development of wastelands and dry lands. Currently the oil from *Jatropha curcas* seeds is used for making biodiesel fuel (Fairless, 2007).

2.26 Nomenclature and Taxonomy

Jatropha curcas L. was first described by Swedish botanist Carl Linnaeus in 1753. It is one of many species of the genus *Jatropha*, a member of the large and diverse *Euphorbiaceae* family. Many of the Euphorbiasare known for their production of phytotoxins and milky white sap. The common name ―spurge‖ refers to the purgative properties of many of these *Euphorbias*. There are 170 known species of *Jatropha*, mostly native to the New World, although 66 species have been identified as originating in the Old World (Heller, 1996). A number of *Jatropha* species are well known and widely cultivated throughout the tropics as ornamental plants. The literature identifies three varieties: Nicaraguan (with larger but fewer fruits), Mexican (distinguished by its less-toxic or non-toxic seed) and Cape Verde. The Cape Verde variety is the one commonly found throughout Africa and Asia.

Jatropha curcas L. has many vernacular names including: Physic nut or purging nut (English), *pinhão manso* or *mundubi-assu* (Brazil), *pourghère* (French), *purgeernoot* (Dutch), *Purgiernuss* (German), *purgeira* (Portuguese), *fagiola d'India* (Italian), *galamaluca* (Mozambique), *habel meluk* (Arab), *safed arand* (Hindi), *sabudam* (Thai), *bagani* (Ivory Coast), *Lapalapa* (Nigeria), *makaen* (Tanzania), *piñoncillo* (Mexico), *tempate* (Costa Rica) and *piñon* (Guatemala) (Brittaine and Lutaladio, 2010).

2.27 Description of *Jatropha curcas*

Jatropha, a succulent perennial shrub or small tree, can attain heights of more than 5 metres, depending on the growing conditions. In each case, the trees are slightly more than two years old. Seedlings generally form a central taproot, four lateral roots and many secondary roots. The leaves, arranged alternately on the stem, are shallowly lobed and vary from 6 to 15 cm in length and width. The leaf size and shape can differ from one variety to another. As with other members of this family, the vascular tissues of the stems and branches contain white latex. The branches and stems are hollow and the soft wood is of little value. *Jatropha* is monoecious, meaning it carries separate male and female flowers on the same plant. There are fewer female than male flowers and these are carried on the apex of the inflorescence, with the more numerous males borne lower down. The ratio of male to female flowers averages 29:1 but this is highly variable and may range from 25-93 male flowers to 1-5 female flowers produced on each inflorescence (Raju and Ezradanum, 2002).

It also has been reported that the male-to-female flower ratio declines as the plant ages (Achten *et al.*, 2010), suggesting that fruiting capacity may increase with age. The unisexual flowers of *Jatropha* depend on pollination by insects, including bees, flies, ants and thrips. One inflorescence will normally produce 10 or more fruits. Fruit set generally results from cross pollination with other individual plants, because the male flowers shed pollen before the female flowers on the same plant are receptive. In the absence of pollen arriving from other trees, *Jatropha* has the ability to self-pollinate, a mechanism that facilitates colonization of new habitats (Raju and Ezradanum, 2002). The fruits are ellipsoidal, green and fleshy, turning yellow and then brown as they age. Fruits are mature and ready to harvest around 90 days after flowering. Flowering and, therefore, fruiting are continuous, meaning that mature and immature

fruits are borne together. Each fruit contains two or three black seeds, around 2 cm × 1 cm in size. On average, the seeds contain 35 percent of non-edible oil. The immature and mature fruits are shown together with the seed. *Jatropha* grows readily from seed which germinate in around 10 days, or from stem cuttings. Growth is rapid. The plant may reach one metre and flower within five months under good conditions (Heller, 1996). The growth is sympodial, with terminal flower inflorescences and lateral branching, eventually reaching a height of 3 to 5 metres under good conditions. It generally takes four to five years to reach maturity (Henning, 2008).*Jatropha* trees are believed to have a lifespan of 30 to 50 years or more.

2.28 Medicinal Properties of *Jatropha curcas*

The therapeutic efficacies of many indigenous plants for various diseases had been described by traditional herbal medicinal practitioners. The past decade has seen a considerable change in opinion regarding ethnopharmcological therapeutic application. The presence of various life sustaining constituents in plants has urged scientist to examine these plants with a view to determine potential properties. *Jatropha curcas* is one of the promising ethnomedicinal plants used in Asia and Africa for solving health problems (Lans, 2001). All parts of the plant are used in traditional medicine and active components are being investigated in scientific trials. Several ingredients appear to have promising applications both in medicine and as a plant protectant.

2.28.1 Folk medicine

According to many reports published, *J. curcas* is a proven folk medicine used by many in almost all parts of the world (Sarkiyayi *et al.,* 2016). The leaves of this plant show antileukemic activity and contain aamyrin, b-sitosterol, stigmasterol, and campesterol, 7-keto-bsitosterol, stigmast-5-ene-3-b, 7-a-diol, and stigmast-5-ene- b, 7 b-diol, isovitecxin and vitecxin (Debnath and Bisen, 2008).

The seed also contains saccharose, raffinose, stachyose, glucose, fructose, galactose, protein and oils largely of oleic- and linoleic- acids, curcasin, arachidic-, linoleic-, myristic-, oleic-, palmitic-, and stearic-acids (Perry, 1980). The plant is a source of folk remedy for alopecia, anasorca, ascites, burns, carbuncles, convulsions, cough, dermatitis, diarrhoea, dropsy, dysentery, dyspepsia, eczema, erysipelas, fever, gonorrhea, hernia, incontinence, inflammation, jaundice, neuralgia, paralysis, parturition, pleurisy, pneumonia, rash, rheumatism, scabies, sciatica, sores, stomachache, malaria fever, syphilis, tetanus, thrush, tumors, ulcers, uterosis, whitlows, yaws, and yellow fever. Seed is viewed as aperient; the seed oil emetic, laxative, purgative, for skin ailments. Seeds are also used for treatment of dropsy, gout, paralysis, and skin ailments (Debnath and Bisen, 2008). It is also prescribed homeopathically for cold sweats, colic, collapse, cramps, cyanosis, diarrhoea and leg cramps. It is also reported to be abortifacient, anodyne, antiseptic, cicatrizant, depurative, diuretic, emetic, hemostat , lactagogue, narcotic, purgative, rubefacient, styptic, vermifuge, and vulnerary. Leaves of physic nut (*Jatropha curcas/gossypifolia*) are boiled and the decoction used to clean sores (Osoniyi and Onajabi, 2003; Debnath and Bisen, 2008). *Jatropha curcas* latex is applied to external wounds in Perú and Indonesia. The leaf bath is used for rash, bewitchment and poultices for sores in Trinidad, *J. curcas* leaf and bark contain glycosides, tannins, phytosterols, flavonoids and steroidal sapogenins (Debnath and Bisen, 2008).

Traditional practitioners from many countries commonly use *J. curcas* for many medical conditions. Mauritians massage ascitic limbs with the oil. Leaves are regarded as antiparasitic, applied to scabies, rheumatism and hard tumors. Cameroon natives apply the leaf decoction in arthritis (Debnath and Bisen, 2008). Colombians drink the leaf decoction for venereal disease.

Bahamans drink the decoction for heartburn. Costa Ricans poultice leaves onto erysipelas and splenosis. Latex applied topically to bee and wasp stings and is also used to dress sores and ulcers and inflamed tongues. Cubans apply the latex to toothache. Colombians apply the latex to burns, haemorrhoids, ringworm, and ulcers. Barbadians use the leaf as tea for marasmus, Panamanians for jaundice. Root is used in decoction as a mouthwash for bleeding gums and toothache. It is also used for eczema, ringworm, and scabies (Debnath and Bisen, 2008). Venezuelans take the root decoction for dysentery. Preparations of all parts of the plant, including seeds, leaves and bark, fresh or as a decoction are used in traditional medicine and for veterinary purposes. The oil has a strong purgative action and is widely used for skin diseases and to soothe pain caused by rheumatism. A decoction of leaves is used against cough and as an antiseptic after birth. Branches are used as a chewing stick in Nigeria (Debnath and Bisen, 2008).

2.28.2 Anti-parasitic activities of *Jatropha curcas*

Traditional uses of plants have paved path for investigations for their bioactive compounds through screening programs. Such studies have helped in the detection of a significant number of therapeutic properties. Parasitological tests were carried out on laboratory bench surfaces using the sap and crushed leaves of *Jatropha curcas* in order to investigate the disinfectant/antiparasitic activities as a first step in providing a cheap, readily available disinfectant and malaria vector control agent. The sap extracted from *J. curcas* was found to exhibit germicidal actions on the growth of common bacteria of *Staphylococcus, Bacillus* and *Micrococcus* species, six hours after initial application. Ova of *Ascaris lumbricoides* and *Necator americanus*incubated in 50% and 100% concentrations of the sap at room temperature showed either no evidence of embryonation after 21 days in the case of *A. lumbricoides*, negation of hatchability in hookworm, or complete distortion in both (Florence *et al.*,2012). The sap also

inhibits growth of *Candida albicans* and *Staphylococcus aureus* (Robineau, 1991). Aqueous extracts of physic nut leaves were effective in controlling *Sclerotium* sp., an *Azolla* fungal pathogen (Garcia and Lavas, 1999). Sarkiyayi *et al.* (2016) reported that the aqueous stem bark extract of *Jatropha curcas* possess some antimalarial properties. The extract showed protective effect on liver tissues and kidney indices. This suggest the possibility of using *Jatropha curcas* extract in the treatment of malaria and perhaps a promising future for this new class of anti bacterial agent could be actualized.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria-Kaduna state, Nigeria. Located in Northwestern region of Nigeria. The Department is on latitudes 11°7', 11°12 N and longitudes 7° 41 E (Mammane*t al.*, 2000), with an altitude of 500-700 meters above sea level and a total area of 300km^2. The climatic characteristics is that of tropical savannah (Mortimore, 1970), with a monthly mean temperature of 25.25°C (ranging from 13.8°C to 36.7°C) and annual rainfall of 1092.8mm (Agbogbu*et al.*, 2006).

3.2 Collection of *Jatropha curcas* Leaf

Healthy leaves of *Jatropha curcas* L. leaf were collected from the *Jatropha* plantation of National Research Institute of Chemical Technology (NARICT) and authenticated at the Herbarium of the Department of Botany, Ahmadu Bello University, Zaria with a voucher No. 01911.The plant was washed thoroughly under running tap water to remove dust and other unwanted particles. The leaves were air dried under shade at room temperature.

3.3 Extraction and Solvent Partitioning

3.3.1 Plant extraction

The dried leaves was crushed to powder with pestle and mortar and five hundred grams of the powder was cold macerated in 1000ml of distilled water for 24 hours with constant shaking and filtered using Whitman's filter paper No. 1. It was then concentrated to dryness on water bath at 40°C and the crude extract was kept in desiccators and dried.Another Five hundred gram (500 g) of the plants powder was cold macerated in 1000 ml of 70 % methanol. The methanol extracts

was periodically shaken for 48hours and filter. The procedure was repeated three times to exhaustively extract the constituents of the plant materials. The filtrate was kept and two-third of the initial solvent was added to the content of funnel, shaken and allowed to stand for another 24hours, after then it was filtered. The filtrate was pooled together and then solvent was remove in vacuum using rotary evaporator to obtained crude methanol extract and kept for the investigation.

3.3.2 Solvent partitioning

The crude methanol extract was then partitioned using solvents of different polarities as described by Handal *et al*. (2008). The solid crude methanol extract was dissolve in distilled water to form an aqueous extract which was then serially partitioned with N-hexane, Ethyl acetate and N-butanol in a separating funnel. Each partition process was repeated three times using equal volume of each solvent and similar fraction were pooled together and concentrated in vacuum at 40°C (Handa *et al*., 2008).

3.4 Phytochemical Screening

Phytochemical screening of the crude plant extract was carried out employing standard procedures and tests described by Trease and Evans, (1983). This is to test for the presence of biologically active compounds that can be extracted like flavonoids, alkaloids, saponins, tannins, Terpenoids, glycosides, volatile oils, active ingredients and reducing sugars.

3.4.1 Test for carbohydrate

Molisch's Test

Three drops of Molisch's reagent was added to about 1g of the aqueous extract in a test tube, follow by addition of concentrated sulphuric and mixed together. The mixture was allowed to

stand for two minutes. The formation of a reddish color ring at the interface indicates the presence of carbohydrate.

3.4.2 Test for flavonoids

i. Shinoda Test

About 2g extract was dissolved in 2 ml of methanol and pieces of magnesium chips wasadded, followed by 2-3 drops of concentrated hydrochloric acid. The formation of a pink, orange or red to purple coloration indicated the presence of flavonoids.

ii. Sodium Hydroxide Test

Two drops of 10% sodium hydroxide solution was added to a solution of the extracts (CME), yellow coloration indicated the presence of flavonoids.

3.4.3 Test for saponin

i. Frothing test

Ten mls of distilled water was added to a small portion of the extract and shake continuously for 30 seconds. The solution was allowed to stand for 5 minutes, the formation of a persistent froth will indicated the presence of saponin.

3.4.4 Test for terpenoids/Steroid

I. Salkowskis test

About 2g of the extract was dissolved in 2ml of chloroform, 3 drops of concentrated sulphuric acid (salkowiskis reagent) was added at the side of the test tube .The appearance of a reddish colouration at the interface indicated the presence of terpenoids.

II. Liebermann–Burchard Test:

To 1 g of the extract equal volume of acetic anhydride was added and mix together gently. 1ml of concentrated sulphuric acid was added down the test tube. An instant colour change was observed within a period of an hour. Blue to blue-green colour at the upper layer and a reddish, pink or purple color at the junction of the two layers indicated the presence of triterpene.

3.4.5 Test for alkaloids

I. Dragendoffs Test

1g of the extract was dissolved in 2ml of 1%aqueous hydrochloric acid with vigorous stirring in a water bath. The mixture was filtered and few drops of Dragendoffs reagent will be added, rose red precipitate indicated the presence of alkaloids.

II. Mayers Test

About 2ml acidic solution of the extracts in a test tube, 3 to 4 drops of Mayers reagent was added, a cream precipitate indicated presence of alkaloids.

3.4.6 Test for free Anthraquinones

2g of the extract was dissolved in 5ml of Chloroform, it was shaked and then filtered. After that, 10% amonia solution was added to the filtrate and was shake vigorously until there is presence of bright pink colour in the aqueous upper layer which indicated the presence of anthraquinone.

3.4.7 Test for Tannins

I. Ferric chloride test

Three drops of ferric chloride solution was added to the solution of the extracts. Blue-black coloration indicated the presence of Tannin.

II. Lead sub-acetate test

Four drops of lead sub- acetate solution was added to 2g of the extract, the formation of a cream color precipitate indicated the presence of tannins.

3.5 Study Animals

Two hundred and twenty five albino mice were bought from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were housed in cages under standard laboratory conditions and fed on standard pelleted diet (growers mash). They were acclimatized to the laboratory environment for 10 days with food and water provided *ad libitum*. Ethical clearance was sought for from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval No. ABUCAUC/2020/67.

3.6 Study Drug

Analytical grade of chloroquine and extract was used in the antiplasmodial study and they are administered orally with the aid of an insulin syringe and needle.

3.7 Malaria Parasite

The malaria parasite (*Plasmodium berghei* NK-65) was obtained from a donor Mousein National Institute for Medical Research (NIMR), Lagos, Nigeria. The parasite was maintained by sub-passaging into healthy mice.

3.8 Acute Toxicity Test

The LD_{50} of the aqueous and methanolic extract of the leaves were tested using modified Lorke, (1983) method to determine safety. The aqueous and methanolic extract of the leaves of *J. curcas* were administered in doses of 1000, 100 and 10 mg/kg body weight to three groups of mice (n = 3) in the first phase. While the second phase concentration were 1600, 2900 and 5000 mg/kg body weight to three groups of mice (n = 3). The procedure was repeated for N-butanol, Hexane and Ethyl Acetate fractions.The mice were kept under same conditions and observed for toxicity signs including change in behavior, loss of appetite and mortality for 24 hours. A total of ninety mice were used for the toxicity test.

3.9 Parasite Inoculation

The inoculation of the parasite was carried out by determining both the percentage parasitemia and erythrocytes count of the donor mouse using a haemocytometer and appropriate dilutions of the infected blood with isotonic saline. Each mouse in all the groups except the group 1 micewere inoculated intraperitoneally with 0.2 ml of infected blood containing about $1x10^7$*Plasmodium berghei* parasitized red blood cells.

3.10 Experimental Design (Animal Grouping)

Ninety Swiss albino mice were divided into eighteen (18) groups of five (5) mice each. Groups 2-18 were inoculated with the rodent malaria parasite *Plasmodium berghei* from the same donor mouse. Groups 4-18 were treated with 250, 500 and 750 mg/Kg body weight of the crude methanolic extract, aqueous extract, n-hexane, n-butanol and Ethyl acetate fractions of *J. curcas* leavesrespectively. Group 3 was treated with 20 mg/Kg body weight of Chloroquine while Groups 1 serve as negative control while group 2 served as positive control (Infected treated with normal saline).

Table 1: **Animal Grouping**

Group No	Experimental Groups	Dosage (mg/kg)
1	Negative Control	Unparasitized + distilled water
2	Positive Control	Infected mice + Distilled water
3	Standard Control	Parasitized + Chloroquine (20mg/kg)
4	Methanolic Extract	Methanolic Extract 250mg/kg
5		Methanolic Extract 500mg/kg
6		Methanolic Extract 750mg/kg
7	Aqueous Extract	Aqueous Extract 250mg/kg
8		Aqueous Extract 500mg/kg
9		Aqueous Extract 750mg/kg
10	N-hexane Fraction	N-hexane Fraction 250mg/kg
11		N-hexane Fraction 500mg/kg
12		N-hexane Fraction 750mg/kg
13	N-butanol Fraction	N-butanol Fraction 250mg/kg
14		N-butanol Fraction 500mg/kg
15		N-butanol Fraction 750mg/kg
16	Ethyl Acetate Fraction	Ethyl Acetate Fraction 250mg/kg
17		Ethyl Acetate Fraction 500mg/kg
18		Ethyl Acetate Fraction 750mg/kg

3.10.1 Evaluation of suppressive activity of the extract (Peter's 4-Day Test)

Peter's 4-day test was used to evaluate the activity of the extracts against early *P. berghei* infection in Mice (Peter *et al.*, 1965). The mice were inoculatedintraperitoneally with 1×10^7 infected erythrocytes.Treatment commenced three hours after mice had been inoculated with the parasite on day 0 and then continued daily for four days from day 0 to day 3with 250, 500 and 750 mg/kg of the crude extract and its fraction orally. Group 3(standard control) was treated daily with 20 mg/kg of chloroquine. After treatment was completed, thin blood film was prepared from the tail of each animal on day 4 to determine parasitemia and also percentage suppression using

$$\%\ \text{Suppression} = \frac{\text{APC} - \text{APT}}{\text{APC}} \times 100$$

Where **APC** = Average Parasitaemia in the Negative Control, **APT** = Average Parasitaemia in the Treated group

3.10.2 Evaluation of curative activity (Rane's test)

Evaluation of the curative potential of the most active dose in Peter's test was carried out as described by Ryley and Peters (1995).*Plasmodium berghei*(NK 65) was injected intraperitoneally into the various groups of mice (n=5) on the first day (Do)except the healthy control. Seventy-two hours later (D3), different doses of the crude extracts/fraction were administered to the mice. Chloroquine and normal saline were given to the control groups. Treatments were administered once daily for five (5) consecutive days. Giemsa stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor parasitemia level.

3.11 Determination of Parasitaemia

Blood were collected from the tail of each mouse every day except for group one, thin blood smears were prepared as described by Gilles (1993). It was prepared by placing the smooth edge of another slide on the blood at an angle of 45°, then the blood was allowed to spread along the entire width of the slide before it was gently smeared on the slide. The slides with smeared blood were air dried and fixed with solute methanol for 10 minutes to preserve the blood cells for a long period. After 10 minutes, the slide was rinsed under running tap water and allowed to dry. After drying, the slide was stain with 5% gimsa stain solution for 3 minutes. The stain was washed off and allowed to dry and subsequently examined under the oil immersion (×100) objectives lens. The percentage and intensity of the parasite were calculated as described by Cheesbrough (2008).

3.12 Haematological Assays

Blood was taken from the eye vein into a bottle at the beginning of the study for full blood count and differential count. At the expiration of treatment period, mice were starved overnight and sacrificed. Blood was collected through cardial punture for haematological parameters. Blood sampleswere collected in EDTA bottle to prevent blood coagulation. Full blood count and differential count were carried out.It has been established that the normal range of RedBlood Cell (RBC) and Packed Cell Volume (PCV) in healthy mice are 9.23-9.42 $\times 10^6$/mm3 and 40.8 – 41.7 g/dL (Schnell *et al.*, 2002).

3.13 Histopathology

Vital body organs of the mice were harvested (liver, intestine, heart and kidney) and immediately preserved in clean sample bottles containing 10% formalin. They were processed for histology examination using the method outlined by Bakare *et al.* (2000). The process involves immersing the organs in 10% formalin, stir gently so that the tissue sample does not stick to that container surface. The tissues were pre-embedding in 70 and 95% ethanol solutions. The tissues were dehydrated in a graded series of ethanol, this was then cleared with a paraffin solvent (Xylene) for 1 hour (two times). The cleared tissue was then infiltrated with paraffin for 1 hour and embedded in paraffin wax. The tissue was sectioned with the aid of a microtome (4-5mm). The de-waxed sections were then stained with hematoxylin and eosin (H and E). Histopathological lesions were then recorded and photomicrographs with a digital camera.

3.14 Gas Chromatography Mass Spectroscopy Analysis (GC-MS)

The crude methanolic extract of *J. curcas* leaves was analyzed in the Central Research Laboratory, Federal University of Technology, Akure (FUTA) on a capillary GC column. Identification were made by observing their retention indices and mass spectra then comparing it to that in the NIST library using a search engine. A small amount of the extract was dissolved in

methanol and then anhydrous sodium sulphate was added. It was filtered with 0.22 micro membrane.

3.14.1 Gas Chromatography Mass Spectroscopy Procedure

GC-MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA) which includes a Perkin Elmer Auto sampler XLGC. The column used was Perkin Elmer Elite - 5 capillary column measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5ml/min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 110°C for 4 min, then an increase to 240°C. And then programmed to increase to 280°C at a rate of 20°C ending with a 5 min. Total run time was 90 min. The MS transfer line was maintained at a temperature of 200°C. The source temperature was maintained at 180°C. GC-MS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of National Institute of Standards and Technology (NIST) library. Measurement of peak areas and data processing were carried out by Turbo-MassOCPTVS-Demo SPL software.

3.15 Data Analyses

The LD_{50} was calculated as the geometric mean of the product of the lowest lethal dose and highest non-lethal dose.

LD50 =√lowest lethal dose× highest non lethal dose

One-way ANOVA was used to determine the activities of the extracts against *P. berghei* and haematological parameters. Mean was separated using least significant difference (LSD). The data was analysed at $P \leq 0.05$ using SPSS (Statistical Package for Social Sciences) version 23.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield of Aqueous and Methanol leaf Extracts of *Jatropha curcas* Leaf and its Fraction

The percentage yield of the aqueous and methanolic fractions of *Jatropha curcas* leafare shown in table 4.1. The percentage yield (w/w) of the various extracts have methanol (59.20%) as the highest yield, followed by aqueous extract and butanol fraction (33.95 and 11.19% each) while ethyl acetate and hexane fraction (10.08 and 8.52 %) have the lowest yield.

4.2 Qualitative Phytochemicals Constituents of Aqueous and Methanol Leaf Extracts and Its Fractions

Qualitative phytochemical constituents of aqueous and methanol extract of *J. curcas* leaf and it fractions were presented in Table 4.2. The results reveal that anthraquinone was absent in all the extract while presence of saponin, flavonoid, alkaloids, tannin, triterpene, carbohydrates, steroids, glycosides and cardiac glycosides were detected in aqueous extract, methanol extract and butanol fraction.

The table also show the presence of alkaloid, steroids, glycoside and cardiac glycosides in hexane fraction. While flavonoid, alkaloids, tannin, triterpene, carbohydrates, steroids, glycosides and cardiac glycosides are presence in Ethyl acetate fraction.

Table 4.1: Percentage Yield of Aqueous and Methanol leaf Extracts of *Jatropha curcas* Leaf and its Fraction

Extracts	Percentage Yield (%)
Aqueous	33.95
Methanol	59.20
Hexane	8.52
Ethyl Acetate	10.08
Butanol	11.19

Table 4.2: Phytochemicals screening of *Jatropha curcas* Leaf Extracts and Fractions

Phytochemicals Constituents	Aqueous	Methanol	Hexane	Ethyl Acetate	Butanol
Anthraquinone	-	-	-	-	-
Saponin	+	+	-	-	+
Flavonoids	+	+	-	+	+
Alkaloids	+	+	+	+	+
Tannin	+	+	-	+	+
Triterpene	+	+	-	+	+
Carbohydrates	+	+	-	+	+
Steroids	+	+	+	+	+
Glycosides	+	+	+	+	+
Cardiac Glycosides	+	+	+	+	+

Key: + Present - Absent

4.3 Mass Spectrometry of Chemical Compounds in Methanol Extract of *Jatropha curcas* Leaf

Gas column mass spectroscopy result revealed the present of seventeen (17) bioactive compounds of which Phytol (31.64%), Octadecanoic acid (18.30%) and 9,12,15-Octadecatrienoic acid (11.20%) are present in appreciable quantity while Benzene, [1-(2,4-cyclopentadien-1- ylidene)ethyl] (0.53%) and Ethyl 9-decenoate (1.25%) are the least (Table 4.3).

4.4 Median Lethal Dose (LD50) Determination of Aqeuous and Methanol Extract of *Jatropha curcas* leaf and its Fractions

Table 4.4 revealed the median lethal dose of aqueous and methanol leaf extracts of *J. curcas* and its fractions. No death was recorded after the oral administration up to a dose of 5000mg/kg body weight for the extracts and its fractions.LD50 was calculated to be ≤5,000 mg/kg.

Table 4.3: The Active Compound Detected in Methanolic Extracts of *Jatropha curcas* Leaf by Gas Column Mass Spectroscopy

S/N	Name of Compound	Molecular Formula	Retention Time	Peak Area
1	Benzene, [1-(2,4-cyclopentadien-1-ylidene)ethyl]	$C_{14}H_{14}S$	7.425	0.53
2	Ethyl 9-decenoate	$C_{12}H_{22}O_2$	17.679	1.25
3	Methyl stearate	$C_{19}H_{38}O_2$	14.990	1.56
4	E-3-Pentadecen-2-ol	$C_{15}H_{30}O$	16.003	1.60
5	2,6-Octadiene	C_8H_{14}	13.233	1.68
6	Benzene-1,2-dicarboxylic acid	$C_8H_6O_4$	8.221	1.87
7	3-Aminothiobenzamide	$C_7H_8N_2S$	11.179	2.17
8	Hexasiloxane	$C_{12}H_{38}O_5Si_6$	16.747	2.85
9	1,4,8-Dodecatriene	$C_{12}H_{18}$	14.664	2.90
10	9-Octadecenamide	$C_{18}H_{35}NO$	17.330	3.34
11	Nonanoic acid	$CH_3(CH_2)_7CO_2H$	15.739	3.99
12	2-Pentadecen-4-yne	$C_{15}H_{26}$	17.628	4.26
13	Linoleic acid	$C_{20}H_{34}O_2$	15.362	4.38
14	Hexadecanoic acid	$C_{10}H_{18}$	12.318	6.48
15	9,12,15-Octadecatrienoic acid	$C_{14}H_{24}O_2$	15.436	11.20
16	Octadecanoic acid	$C_{12}H_{14}0_4$	13.325	18.30
17	Phytol	$C_{20}H_{40}O$	14.818	31.64

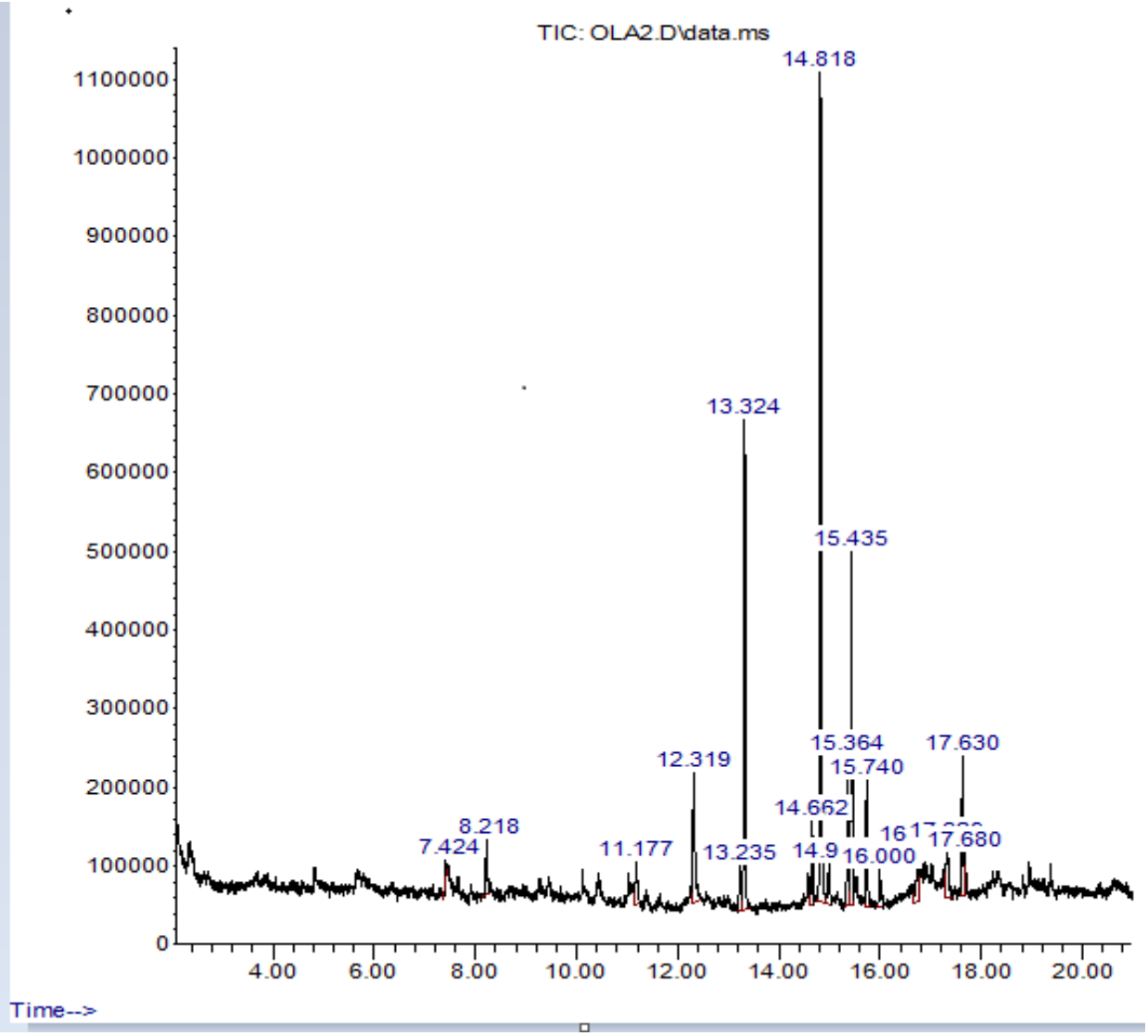

Fig. 4.1: Spectrum of the Most Abundant Active Ingredients in the Methanol Leave Extract of *Jatropha curcas*

Table 4.4: Median Lethal Dose (LD_{50}) Determination for Aqeuous and Methanol Extract of *Jatropha curcas* Leaf and its Fractions

Phase	Dosage of leaf extract/fractions (mg/kg)	No. of Death/No of Mice Used	% Mortality
I	10	0/3	0.0
	100	0/3	0.0
	1000	0/3	0.0
II	1600	0/3	0.0
	2900	0/3	0.0
	5000	0/3	0.0

4.5 Suppressive Antiplasmodial Activity of Aqueous and Methanol Extracts of *Jatropha curcas* Leaf and it Fractions on Mice Infected with *Plasmodium berghei*

At the end of the four (4) days suppressive test, the extracts and its fraction showed a dose dependent and significant ($P<0.05$) decrease in parasitaemia level of which methanol extracts and butanol fraction at 750mg/kg exhibited the highest significant percentage suppression (67.15 and 62.15%) compared to the other extract/fraction treated group while chloroquine (20 mg/kg) showed 90.87% suppression.

4.6 Effects of Aqueous and Methanolic extracts of *Jatropha curcas* Leaf and its Fractions on Haematological Parameters of *Plasmodium berghei* Infected Mice on 4-day Suppression Test

The results showed that mean PCV, RBC and WBC in the positive group was significantly ($P\leq0.05$) lower than the treated groups.Methanol extracts showed highest activities at 500 mg/kg of PCV, RBC and WBC (49.44±3.28, 12.78±0.15 and 10.93±0.03) compared to the negative group with mean PCV, RBC and WBC with 33.68±2.18, 8.02±0.73 and 8.94±0.88 respectively.

4.7 Effects of Aqueous and Methanol Extracts of *Jatropha curcas* leaves and it fractions on differential count of *Plasmodium berghei* in infected Mice on 4-day suppression test

The results revealed that neutrophil count was higher in normal control group (55.00±1.53) than infected normal saline and infected treated group. The table also revealed that the lymphocyte of normal control group was lower compared to the infected normal saline and infected treated group. It was also noted that Basophil level of infected normal saline group was higher than other group.

Table 4.5: 4-Days Suppressive Effects of Aqueous and Methanol Leaf Extracts of *Jatropha curcas* and Its Fractions against *Plasmodium berghei* infected Mice

Treatment/ Dose	Aqueous Extract		Methanol Extract		Ethyl Acetate Fraction		Hexane Fraction		Butanol Fraction	
	Parasitaemia Level	% Suppression	Parasitaemia Level	% Suppression	Parasitaemia Level	% Suppression	Parasitaemia Level	% Suppression	Parasitaemia Level	% Suppression
NC	0.00 ± 0.00^{a}	0	0.00 ± 0.00^{a}	0	0.00 ± 0.00^{a}	0	0.00 ± 0.00^{a}	0	0.00 ± 0.00^{a}	0
PC	10.41 ± 0.45^{f}	0	10.41 ± 0.45^{f}	0	10.41 ± 0.45^{f}	0	10.41 ± 0.45^{f}	0	10.41 ± 0.45^{f}	0
IM+CQ (20mg/kg)	0.95 ± 0.12^{b}	90.87	0.95 ± 0.12^{b}	90.87	0.95 ± 0.12^{b}	90.87	0.95 ± 0.12^{b}	90.87	0.95 ± 0.12^{b}	90.87
IM+250mg/kg	4.87 ± 0.07^{de}	53.21	4.30 ± 0.23^{d}	58.69	4.67 ± 0.22^{bcd}	55.14	5.01 ± 0.14^{b}	51.87	4.75 ± 0.25^{bcd}	54.37
IM+500mg/kg	4.20 ± 0.19^{d}	59.65	4.09 ± 0.32^{bc}	60.71	4.39 ± 0.20^{bcd}	57.82	4.92 ± 0.44^{b}	52.73	4.20 ± 0.10^{bcd}	59.65
IM+750mg/kg	4.09 ± 0.32^{bc}	60.71	3.42 ± 0.34^{c}	67.14	4.02 ± 0.02^{de}	61.38	4.28 ± 0.36^{bcd}	58.88	3.94 ± 0.14^{de}	62.15

Key: Values are Mean±SD. Mean with different superscript along the columns are significantly different ($p<0.05$). PC=Positive Control, NC= Negative Control CQ=Chloroquine, IM=Infected Mice

Table 4.6: Haematology of Infected Mice Treated with *Jatropha curcas* crude Extract and Fractions in Peters 4-Days Suppressive Test

Treatment	Dose (mg/kg)	PCV (%)	RBC×10^9/dl	WBC×10^6
NC	-	52.28±2.31^{a}	12.99±0.48^{a}	12.33±0.83^{a}
PC	-	33.68±2.18^{c}	8.02±0.73^{c}	8.94±0.88^{d}
CQ	20	50.33±3.38^{a}	12.86±0.48^{ab}	11.01±0.08^{bc}
Aqueous Extract	250	46.00±1.20^{bc}	11.40±0.06^{ab}	10.80±0.27^{bc}
	500	45.13±0.67^{bc}	11.27±0.24^{abc}	10.03±0.12^{bc}
	750	45.62±1.53^{bc}	11.53±0.87^{ab}	9.90±0.15^{bc}
Methanol Extract	250	40.09±0.58^{bc}	10.87±0.03^{abc}	10.70±0.46^{bc}
	500	49.44±3.28^{b}	12.73±0.15^{ab}	10.93±0.03^{bc}
	750	49.03±0.67^{b}	11.87±0.12^{abc}	10.67±0.18^{bc}
Ethyl Acetate Fraction	250	44.92±1.45^{bc}	12.87±0.61^{bc}	10.57±0.29^{bc}
	500	44.18±3.71^{bc}	11.79±1.29^{abc}	10.43±0.33^{bc}
	750	45.00±0.67^{b}	11.42±0.27^{ab}	9.75±0.0.37^{c}
Hexane Fraction	250	46.87±1.73^{bc}	10.60±0.09^{ab}	9.74±0.39^{c}
	500	44.83±0.33^{b}	10.43±0.33^{ab}	9.77±0.54^{c}
	750	44.02±0.58^{bc}	10.93±0.23^{ab}	10.07±0.44^{bc}
Butanol Fraction	250	49.62±1.16^{bc}	11.17±0.31^{bc}	10.47±0.12^{bc}
	500	44.27±2.03^{bc}	11.88±0.47^{bc}	10.53±0.42^{bc}
	750	45.15±1.45^{bc}	11.23±0.15^{abc}	10.47±0.24^{bc}

Key: Values are Mean±SD. Mean with different superscript along the columns are significantly different ($p<0.05$). PC= Positive Control NC= Negative Control, CQ=Chloroquine

Table 4.7 Differential Count of Infected Mice Treated with *Jatropha curcas* crude Extract and Fractions in Peters 4-Days Suppressive Test

Treatment	Dose (mg/kg)	Neutrophil	Lymphocyte	Basophil	Eosinophil
NC	-	55.00 ± 1.53^{a}	41.33 ± 1.76^{d}	0.00 ± 0.00^{e}	1.67 ± 0.88^{ab}
PC	-	39.00 ± 3.6^{bcd}	53.33 ± 2.96^{abcd}	3.00 ± 0.58^{a}	1.00 ± 0.58^{b}
CQ	20	47.67 ± 1.20^{ab}	48.33 ± 1.20^{cd}	0.33 ± 0.33^{de}	1.33 ± 0.33^{ab}
Aqueous Extract	250	40.00 ± 4.58^{bcd}	60.00 ± 2.52^{abc}	1.00 ± 0.00^{bcde}	3.33 ± 0.88^{a}
	500	39.00 ± 4.34^{bcd}	55.33 ± 4.10^{abc}	1.00 ± 0.00^{bcde}	3.00 ± 0.58^{ab}
	750	43.00 ± 4.36^{abc}	48.67 ± 3.76^{cd}	1.33 ± 0.33^{bcde}	3.33 ± 0.88^{a}
Methanol Extract	250	28.33 ± 2.85^{d}	66.33 ± 2.73^{a}	0.33 ± 0.33^{de}	2.33 ± 0.88^{ab}
	500	41.33 ± 1.33^{bcd}	52.33 ± 2.03^{bcd}	1.33 ± 0.33^{bcde}	3.33 ± 0.88^{a}
	750	30.67 ± 4.41^{cd}	62.67 ± 3.53^{ab}	0.67 ± 0.67^{cde}	3.00 ± 0.00^{ab}
Ethyl Acetate Fraction	250	38.00 ± 4.16^{bcd}	56.33 ± 3.28^{abc}	1.67 ± 0.33^{abcd}	2.67 ± 0.88^{ab}
	500	33.00 ± 2.89^{cd}	60.33 ± 2.52^{abc}	2.33 ± 0.33^{ab}	1.67 ± 0.33^{ab}
	750	32.00 ± 6.66^{cd}	61.33 ± 6.49^{abc}	1.33 ± 0.33^{bcde}	3.00 ± 0.58^{a}
Hexane Fraction	250	37.67 ± 6.36^{bcd}	56.00 ± 7.02^{abc}	1.33 ± 0.33^{bcde}	2.67 ± 0.88^{ab}
	500	37.33 ± 3.67^{bcd}	57.67 ± 2.33^{abc}	1.00 ± 0.58^{bcde}	3.33 ± 0.33^{a}
	750	38.33 ± 6.22^{bcd}	57.33 ± 6.89^{abc}	0.33 ± 0.33^{de}	2.33 ± 0.33^{ab}
Butanol Fraction	250	34.00 ± 4.36^{bcd}	60.67 ± 4.33^{abc}	1.33 ± 0.33^{bcde}	2.00 ± 0.58^{ab}
	500	44.67 ± 0.33^{abc}	49.33 ± 1.45^{bcd}	2.00 ± 0.58^{abc}	2.00 ± 0.00^{ab}
	750	39.33 ± 3.93^{bcd}	55.67 ± 3.84^{abc}	1.00 ± 0.58^{bcde}	2.00 ± 0.00^{ab}

Key: Values are Mean±SD. Mean with different superscript along the columns are significantly different ($p<0.05$). PC= Positive Control NC= Negative Control, CQ=Chloroquine

4.8 Effect of Aqueous and Methanol leaf extracts of *Jatropha curcas* Leaf and it's Fractions on Weight of *Plasmodium berghei* infected Mice on 4-Days Suppression Test

The effects of aqueous and methanol leaf extracts of *J. curcas* and its fractions on weight of *Plasmodium berghei* revealed that the mean weight of positive group were lower than the infected treated but it is not statistically significant (P>0.05).

4.9: The effect of Aqueous and Methanolic Extracts of *Jatropha curcas* leaf and it fractions on Temperature of *Plasmodium berghei* infected mice on 4-days suppression test

The effects of aqueous and methanol leaf extracts of *J. curcas* and its fractions on temperature of *Plasmodium berghei*indicated that the mean body temperature of the infected normal saline groups increased whereas the extracts prevents the increament in the temperature of the infected treated groups but it is not statistically significant (P>0.05).

Table 4.8: The effect of Aqueous and Methanolic Extracts and it Fractions of *Jatropha curcas* leaf on Percentage Weight of *Plasmodium berghei* infected mice on 4-days suppression test

		WEIGHT		
Treatments	**Dose (mg/kg)**	$Day_{initial}$	Day_{final}	% Change
NC	-	20.90 ± 1.69^{b}	22.33 ± 0.67^{bc}	6.84
PC	-	24.67 ± 1.67^{ab}	21.67 ± 0.67^{c}	12.16
CQ	20	24.33 ± 0.88^{ab}	25.03 ± 1.16^{a}	2.87
Aqueous Extract	250	24.63 ± 1.28^{ab}	24.67 ± 0.29^{a}	0.16
	500	22.90 ± 0.49^{ab}	23.80 ± 0.44^{ab}	3.93
	750	22.67 ± 1.20^{ab}	23.27 ± 0.73^{abc}	2.65
Methanol Extract	250	23.33 ± 0.88^{ab}	23.93 ± 0.12^{ab}	2.57
	500	24.37 ± 1.10^{ab}	24.47 ± 0.63^{ab}	0.41
	750	24.90 ± 0.21^{a}	24.67 ± 0.29^{a}	0.92
Ethyl Acetate Fraction	250	22.33 ± 1.20^{ab}	23.37 ± 0.63^{abc}	4.34
	500	22.73 ± 1.37^{ab}	23.00 ± 0.69^{abc}	1.19
	750	23.00 ± 0.58^{ab}	23.47 ± 0.57^{abc}	2.04
Hexane Fraction	250	21.73 ± 0.27^{ab}	23.60 ± 0.70^{abc}	8.61
	500	22.33 ± 0.88^{ab}	23.53 ± 0.35^{abc}	5.37
	750	23.23 ± 1.43^{ab}	24.30 ± 0.40^{ab}	4.61
Butanol Fraction	250	24.23 ± 0.63^{ab}	23.30 ± 0.89^{abc}	3.84
	500	23.07 ± 1.28^{ab}	23.93 ± 0.52^{ab}	3.73
	750	22.00 ± 1.53^{ab}	22.30 ± 0.95^{bc}	1.36
P-value		0.461	0.057	0.431

Key: Values are Mean±SD. Mean with different superscript across the row are significantly different ($p<0.05$). PC=Positive Control NC= Negative Control CQ=Chloroquine

Table 4.9: The effect of Methanolic extracts and it Fractions of *Jatropha curcas* leaves on Temperature of *Plasmodium berghei* infected mice on 4-day suppression test

		Temperature				
Treatments	**Dose (mg/kg)**	T_0	T_1	T_2	T_3	T_4
NC	-	37.70 ± 0.20^{bc}	37.33 ± 0.79^{abcd}	37.33 ± 0.33^{abcd}	37.13 ± 0.41^{ab}	37.50 ± 0.29^{a}
PC	-	38.03 ± 0.09^{ab}	38.10 ± 0.06^{ab}	38.37 ± 0.33^{a}	38.20 ± 0.27^{a}	38.56 ± 0.02^{a}
CQ	20	38.07 ± 0.35^{ab}	37.97 ± 0.07^{ab}	37.17 ± 0.17^{abcd}	37.30 ± 0.25^{ab}	36.83 ± 0.17^{a}
Aqueous Extract	250	37.47 ± 0.45^{bc}	38.30 ± 0.65^{a}	37.87 ± 0.41^{ab}	37.47 ± 0.29^{ab}	37.57 ± 0.67^{a}
	500	37.33 ± 0.26^{bc}	37.37 ± 0.32^{abcd}	37.43 ± 0.24^{abcd}	37.30 ± 0.30^{ab}	37.17 ± 0.23^{a}
	750	37.20 ± 0.40^{bc}	37.67 ± 0.38^{abcd}	36.47 ± 0.75^{cd}	36.60 ± 0.40^{b}	36.57 ± 0.32^{a}
Methanol Extract	250	37.27 ± 0.47^{bc}	37.37 ± 0.58^{abcd}	37.27 ± 0.37^{abcd}	37.03 ± 0.77^{b}	36.73 ± 0.75^{a}
	500	37.37 ± 0.15^{bc}	37.10 ± 0.49^{abcd}	37.13 ± 0.13^{abcd}	36.87 ± 0.13^{b}	36.77 ± 0.34^{a}
	750	36.90 ± 0.15^{c}	37.30 ± 0.25^{abcd}	37.37 ± 0.32^{abcd}	37.17 ± 0.47^{ab}	37.30 ± 0.35^{a}
Ethyl Acetate Fraction	250	37.77 ± 0.38^{abc}	37.23 ± 0.39^{abcd}	36.90 ± 0.47^{bcd}	36.73 ± 0.22^{b}	36.97 ± 0.09^{a}
	500	38.13 ± 0.09^{ab}	37.70 ± 0.35^{abc}	37.40 ± 0.31^{abcd}	37.47 ± 0.24^{ab}	36.73 ± 0.76^{a}
	750	37.67 ± 0.34^{abc}	37.67 ± 0.18^{abcd}	37.13 ± 0.38^{abcd}	37.10 ± 0.21^{ab}	37.10 ± 0.10^{a}
Hexane Fraction	250	37.30 ± 0.45^{bc}	37.97 ± 0.09^{ab}	36.30 ± 0.51^{d}	36.93 ± 0.07^{b}	37.03 ± 0.15^{a}
	500	36.93 ± 0.15^{c}	36.40 ± 0.45^{d}	37.00 ± 0.58^{bcd}	37.33 ± 0.29^{ab}	37.13±0.49a
	750	37.77 ± 0.03^{abc}	37.00 ± 0.06^{bcd}	37.30 ± 0.25^{abcd}	37.13 ± 0.24^{ab}	36.37 ± 0.47^{a}
Butanol Fraction	250	38.70 ± 0.06^{a}	37.37 ± 0.19^{abcd}	37.03 ± 0.03^{bcd}	36.63 ± 0.27^{ab}	36.83 ± 0.17^{a}
	500	38.03 ± 0.35^{ab}	37.87 ± 0.13^{abc}	37.70 ± 0.15^{abc}	36.87 ± 0.13^{b}	37.00 ± 0.06^{a}
	750	37.60 ± 0.31^{bc}	37.80 ± 0.12^{abc}	37.37 ± 0.19^{abcd}	37.47 ± 0.27^{ab}	37.53 ± 0.27^{a}
P-value		0.013	0.080	0.120	0.216	0.761

Key: Values are Mean±SD. Mean with different superscript along the columns are significantly different ($p<0.05$).

PC=Positive Control NC= Negative Control CQ=Chloroquine

Figure 4.2 Curative Activities of Methanol extracts and Butanol Fractions of *Jatropha curcas* leaf on *Plasmodium berghei* infected Mice

The fractions and extracts with the highest antimalarial activities (Methanol and Butanol) was further evaluated for its effect on *Plasmodium berghei*. The result for the curative anti-plasmodial activity of the extracts and its fractions at different concentrations of the plants were represented in fig. 4.2 in which significant increase in parasiteamia level of positive control was observed. However, a significant ($P<0.05$) decrease in parasitaemia level of infected treated groups in a dose dependent manners are observed. Chloroquine group showed the highest activities compared to methanol extracts and Butanol fraction. They all have significant difference compare to infected normal saline group ($P<0.05$).

Table 4.10: Haematology of Infected Mice Treated with Methanol extract and Butanol Fraction of *Jatropha curcas* Leaf in Rane Curative Test

The hematological parameters of the infected mice treated with methanol extracts and butanol fraction of *J. curcas* are indicated in table 4.10, in which the PCV, RBC and WBC of the positive control group significantly decreases compared to treated groups. Methanol extracts showed highest activities at 500 mg/kg of PCV, RBC and WBC (46.71±2.03, 12.41±0.23 and 12.41±0.64) compared to the negative group with mean PCV, RBC and WBC of 36.47±1.86, 7.01±1.25 and 8.63±0.43 respectively.

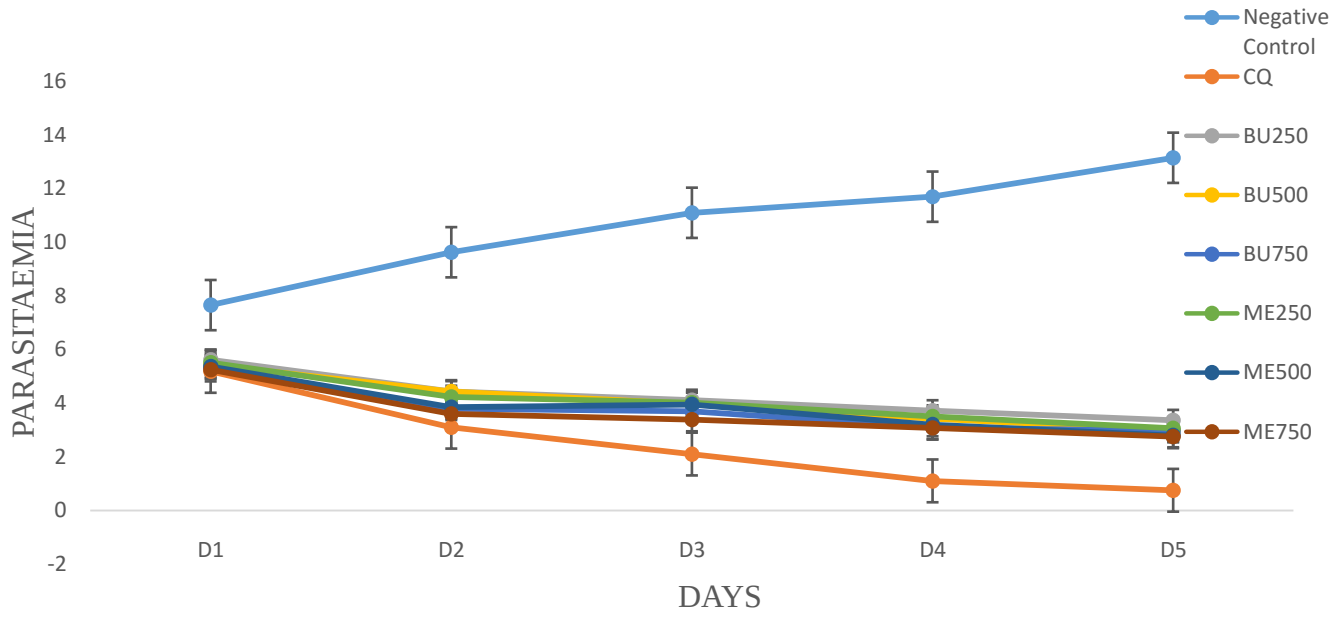

Fig. 4.2: *In Vivo* Curative Antiplasmodial Activity of Crude Methanol and Butanol Fraction of *Jatropha curcas* Leaves against *Plasmodium berghei* infection in Mice

Note

CQ=Chloroquine(mg/kg)

BU= Butanol (mg/kg)

ME= Methanol(mg/kg)

Table 4.10: Haematology of Infected Mice Treated with Methanol extract and Butanol Fraction of *Jatropha curcas* Leaf in Curative Test

Extract	Dose (mg/kg)	PCV (%)	RBC×10^9/dl	WBC×10^6
NC	-	49.88±1.45^{a}	13.10±0.56^{abc}	11.67±0.38^{a}
PC	-	36.47±1.86^{bc}	7.01±1.25^{a}	8.63±0.43^{b}
CQ	20	48.01±2.33^{ab}	12.37±0.62^{abcd}	12.70±0.25^{a}
Butanol Fraction	250	42.67±0.67^{ab}	10.11±0.09^{a}	9.47±1.02^{b}
	500	45.33±1.45^{bc}	11.27±0.75^{abcd}	11.33±0.17^{a}
	750	44.33±2.08^{ab}	11.57±0.12^{abc}	11.10±0.21^{a}
Methanol Extract	250	46.33±1.86^{c}	11.41±0.65^{abcd}	11.53±0.78^{a}
	500	46.71±2.03^{ab}	12.41±0.23^{abcd}	12.07±0.64^{a}
	750	46.00±2.40^{ab}	12.03±0.62^{ab}	11.47±0.37^{a}
P-Value		0.021	0.001	0.001

Key: Values are Mean±SD. Mean with different superscript down the columns are significantly different ($P<0.05$). PC= Positive Control NC= Negative Control CQ=Chloroquine

4.3: Differential Count of Infected Mice Treated with Methanol Extract and Butanol Fraction of *Jatropha curcas* Leaf in Curative Test

The highest number of basophil are recorded in positive group compared to other treated groups and standard control group recorded the highest number of lymphocyte. Methanol extract groups treated with 250 mg/kg recorded the highest number of eosinophil (3.33). All the parameters tested are not statistically significant ($P<0.05$).

4.11: Effects of Methanol Extracts and Butanol Fractions of *Jatropha curcas* leaves on Weight of Mice infected *Plasmodium berghei*

The effects of the weight is revealed in Table 4.12, in which the weight of negative control and chloroquine group increased significantly compared to extracts/fraction treated groups. Also the weight of mice in extract treated group increase in a dose dependent compared to the weight of positive control.

4.12: Effects of Methanol Extracts and Butanol Fractions of *Jatropha curcas* leaves on Temperature of Mice Infected with *Plasmodium berghei*

The effects of the extracts on temperature is revealed in table 4.13 in which the temperature of the positive control group increases compared to the infected treated group and normal control. All the parameters are not statistically significant ($P>0.05$).

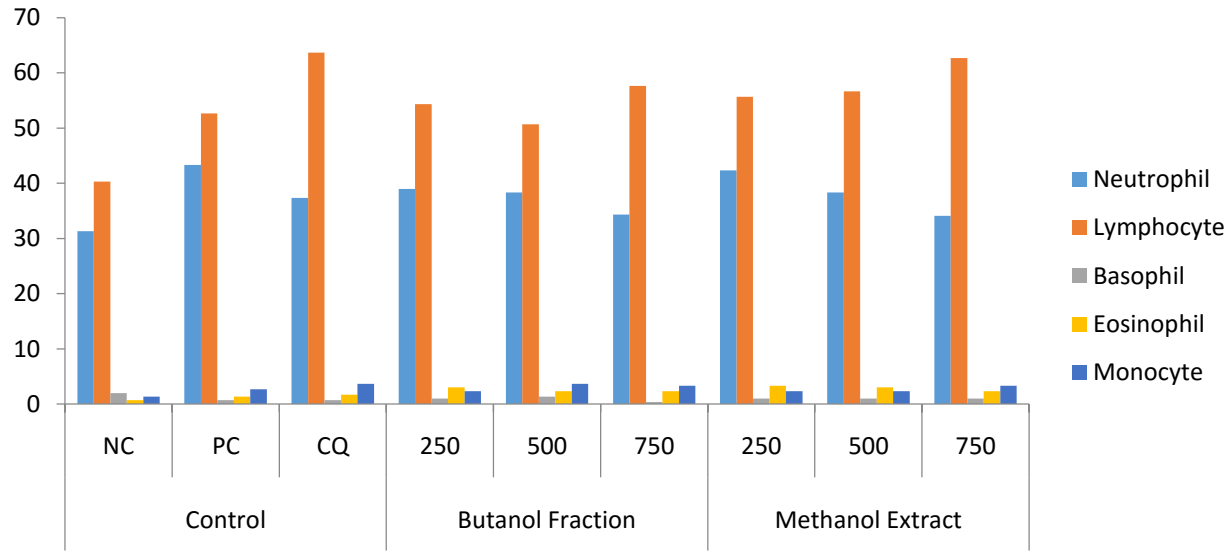

Fig. 4.3: Differential Count of Infected Mice Treated with Methanol Extract and Butanol Fraction of *Jatropha curcas* Leaf in Curative Test

Table 4.11: Effects of Methanol Extracts and Butanol Fraction of *Jatropha curcas*leave on Percentage Weight of Mice Infected with *Plasmodium berghei*

		Weight		
Treatments	Dose (mg/kg)	$Day_{Initial}$	Day_{Final}	% Weight Change
NC	-	25.00±0.58^{a}	30.67±0.33^{a}	22.68
PC	-	25.67±0.88^{a}	21.56±0.58^{bc}	16.05
CQ	20	26.67±0.88^{a}	27.09±0.47^{b}	1.57
Butanol Fraction	250	26.33±1.33^{a}	25.03±0.33^{b}	4.94
	500	24.33±1.20^{a}	22.67±0.33^{c}	6.82
	750	24.33±0.88^{a}	23.01±0.33^{bc}	5.43
Methanol Extract	250	26.33±1.20^{a}	24.43±0.33^{b}	7.22
	500	25.67±1.33^{a}	24.03±0.58^{b}	6.39
	750	25.67±1.20^{a}	24.22±0.88^{b}	5.65
P-value		0.754	0.000	0.049

Key: Values are Mean±SD. Mean with different superscript along the row are significantly different ($P<0.05$). PC= Positive Control NC= Negative Control CQ=Chloroquine

Table 4.12: Effects of Methanol Extracts and Butanol Fractions of *Jatropha curcas* leave on Temperature of Mice Infected with *Plasmodium berghei*

		Temperature				
Group	Dose (mg/kg)	T_0	T_1	T_2	T_3	T_4
IM+Normal Saline	-	37.17±0.17^{a}	36.67±0.17^{a}	37.00±0.00^{abc}	37.33±0.17^{b}	38.27±0.24^{a}
NC	-	36.57±0.22^{a}	36.83±0.17^{a}	37.00±0.29^{abc}	38.33±0.24^{a}	37.90±0.21^{a}
CQ	20	36.80±0.21^{a}	36.53±0.55^{a}	36.63±0.19^{abc}	36.83±0.17^{bc}	36.90±0.21^{b}
Butanol Fraction	250	36.60±0.31^{a}	37.07±0.35^{a}	37.73±0.23^{a}	36.83±0.17^{bc}	36.83±0.33^{b}
	500	36.83±0.17^{a}	37.17±0.17^{a}	36.33±0.19^{bc}	36.60±0.38^{bc}	36.83±0.17^{b}
	750	37.00±0.00^{a}	36.93±0.12^{a}	37.40±0.49^{ab}	36.33±0.44c	37.00±0.29^{ab}
Methanol Extract	250	36.83±0.09^{a}	36.90±0.42^{a}	37.33±0.44^{abc}	37.00±0.29^{bc}	37.33±0.38^{ab}
	500	36.57±0.27^{a}	36.97±0.32^{a}	36.17±0.67^{c}	36.67±0.17^{bc}	36.83±0.38^{b}
	750	36.73±0.38^{a}	37.17±0.17^{a}	37.00±0.29^{abc}	37.17±0.17^{bc}	36.83±0.44^{b}
P-value		0.517	0.839	0.107	0.002	0.239

Key: Values are mean±SD. Mean with different superscript down the columns are significantly different (P<0.05). PC= Positive Control NC= Negative Control CQ=Chloroquine

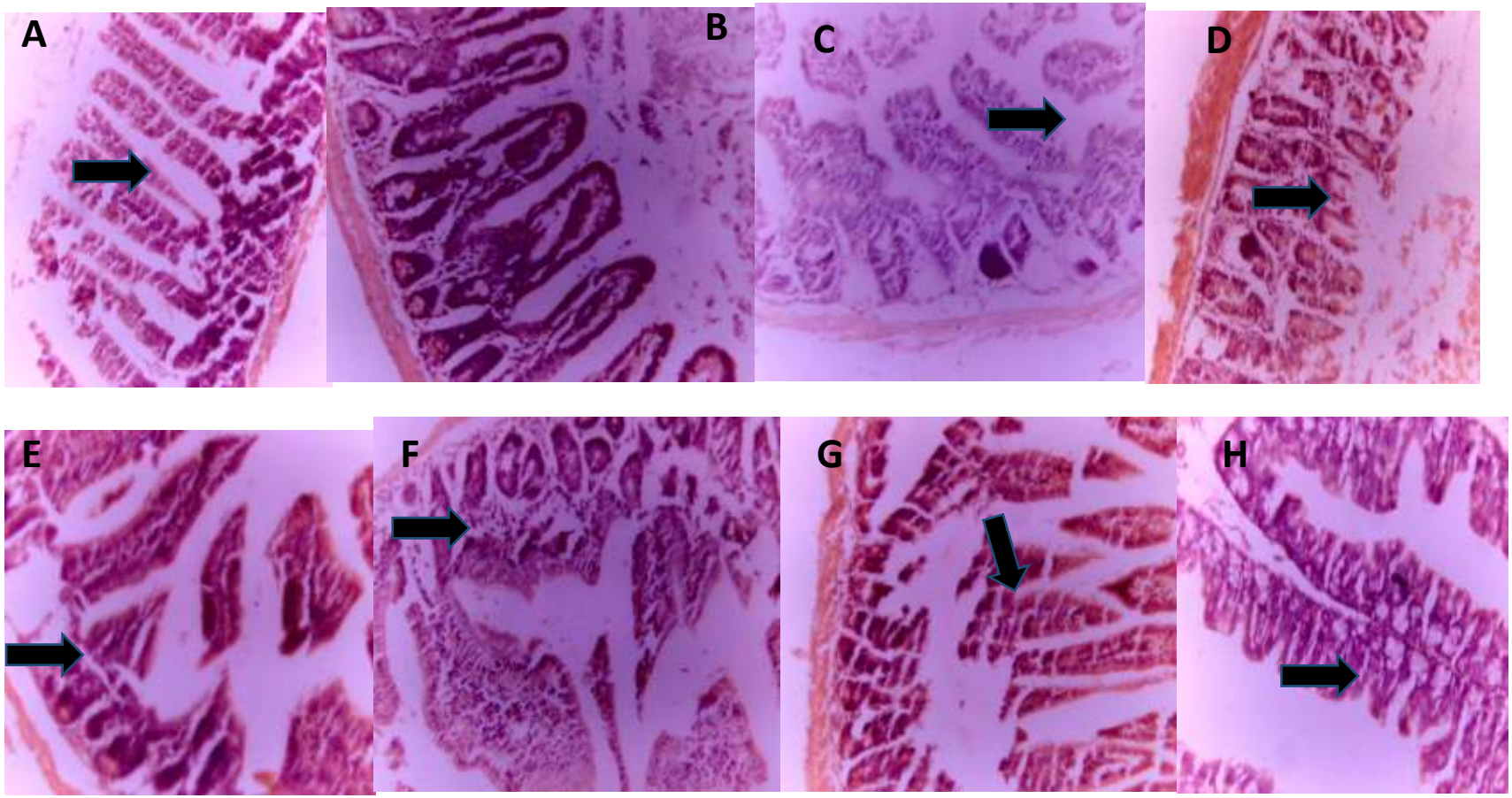

Plate I: (A-H): Photomicrograph of Intestine sections of Mice Haematoxylin and Eosin Stain **×400**

A: Negative Control showing slight necrosis, B: treated with chloroquine showing normal intestine with no inflammation (20mg/kg), C: treated with treated with methanol extracts 250mg/kg showing lymphocyte hyperplasia, D and E: treated with methanol extracts 500 and 750mg/kg showing villi necrosis, F: treated with butanol fraction 250mg/kg showing villi atrophy, G: treated with butanol fraction 500mg/kg showing necrosis H: treated with butanol fraction 750mg/kg showing hydrophobic necrosis

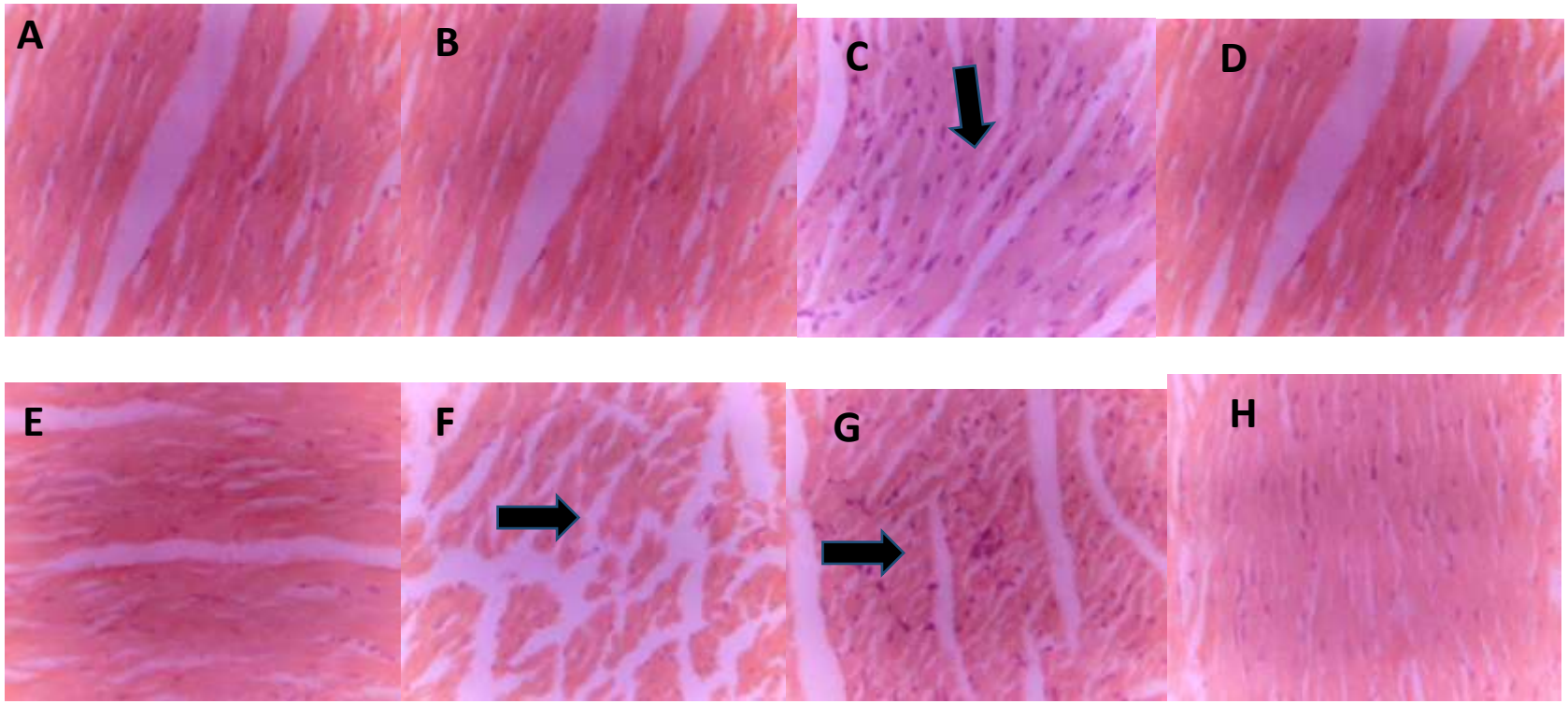

Plate II: Photomicrograph of Heart sections of Mice Haematoxylin and Eosin Stain×400

A: Negative Control showing normal myocardium, B: treated with chloroquine (20mg/kg) shows normal myocardium, C: treated with treated with methanol extracts 250mg/kg showing slight necrosis, D and E: treated with methanol extracts 500 and 750mg/kg showing normal myocardium, F and G: treated with butanol fraction 250 and 500mg/kg showing necrosis, H: treated with butanol fraction 750mg/kg showing normal myocardium

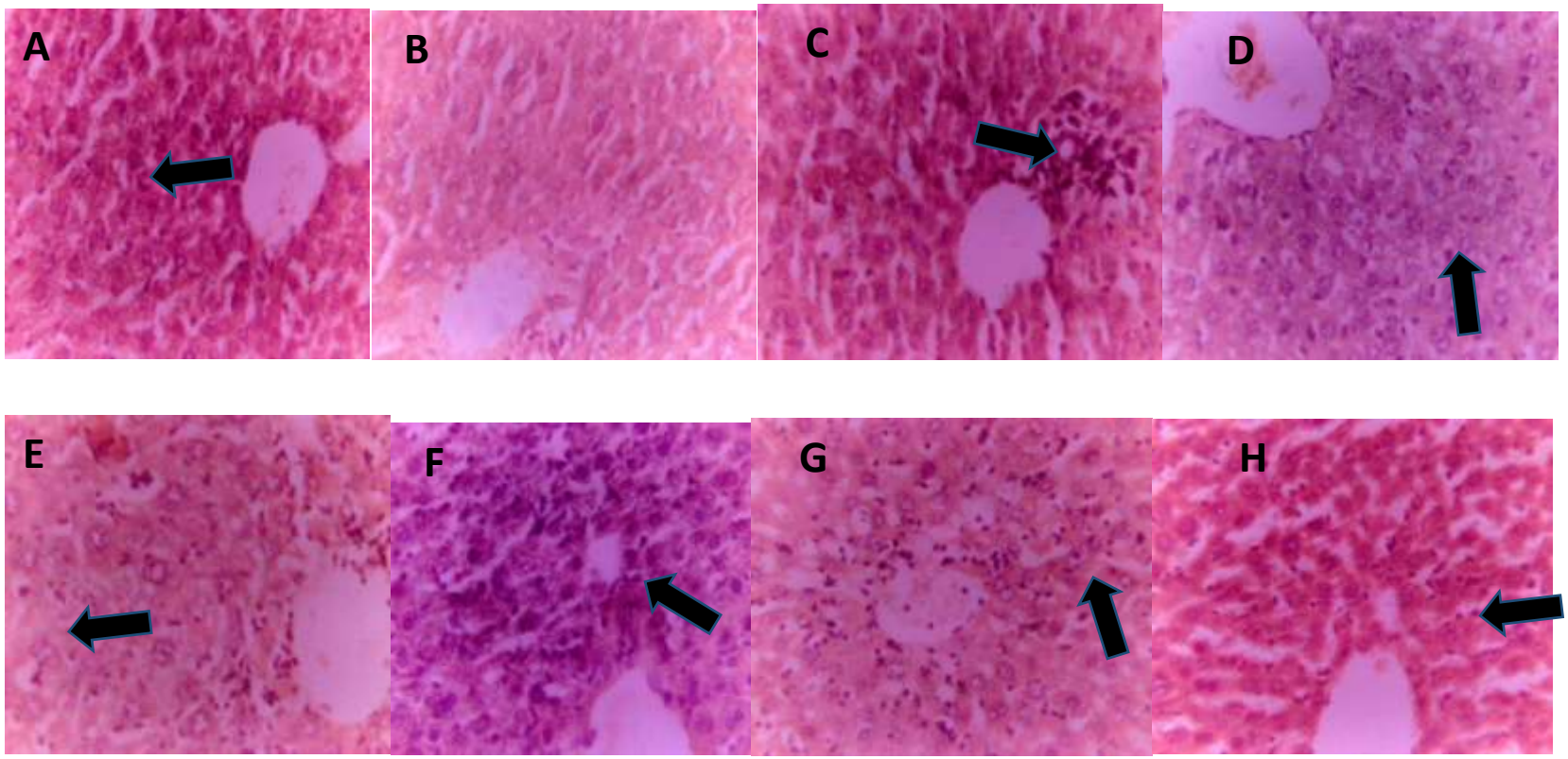

Plate III: Photomicrograph of Liver sections of Mice Haematoxylin and Eosin Stain×400

A: Negative Control showing lymphocyte hyperplasia, B: treated with chloroquine (20mg/kg) showing normal liver C: treated with treated with methanol extracts 250mg/kg showing lymphocyte hyperplasia, D: treated with methanol extracts 500mg/kg showing hepatic necrosis, E: treated with methanol 750mg/kg showing necrosis F: treated with butanol fraction 250mg/kg showing vacoulation and necrosis, G: treated with butanol fraction 500mg/kg showing lymphocyte hyperplasis H: treated with butanol fraction 750mg/kg showing vacoulation and necrosis

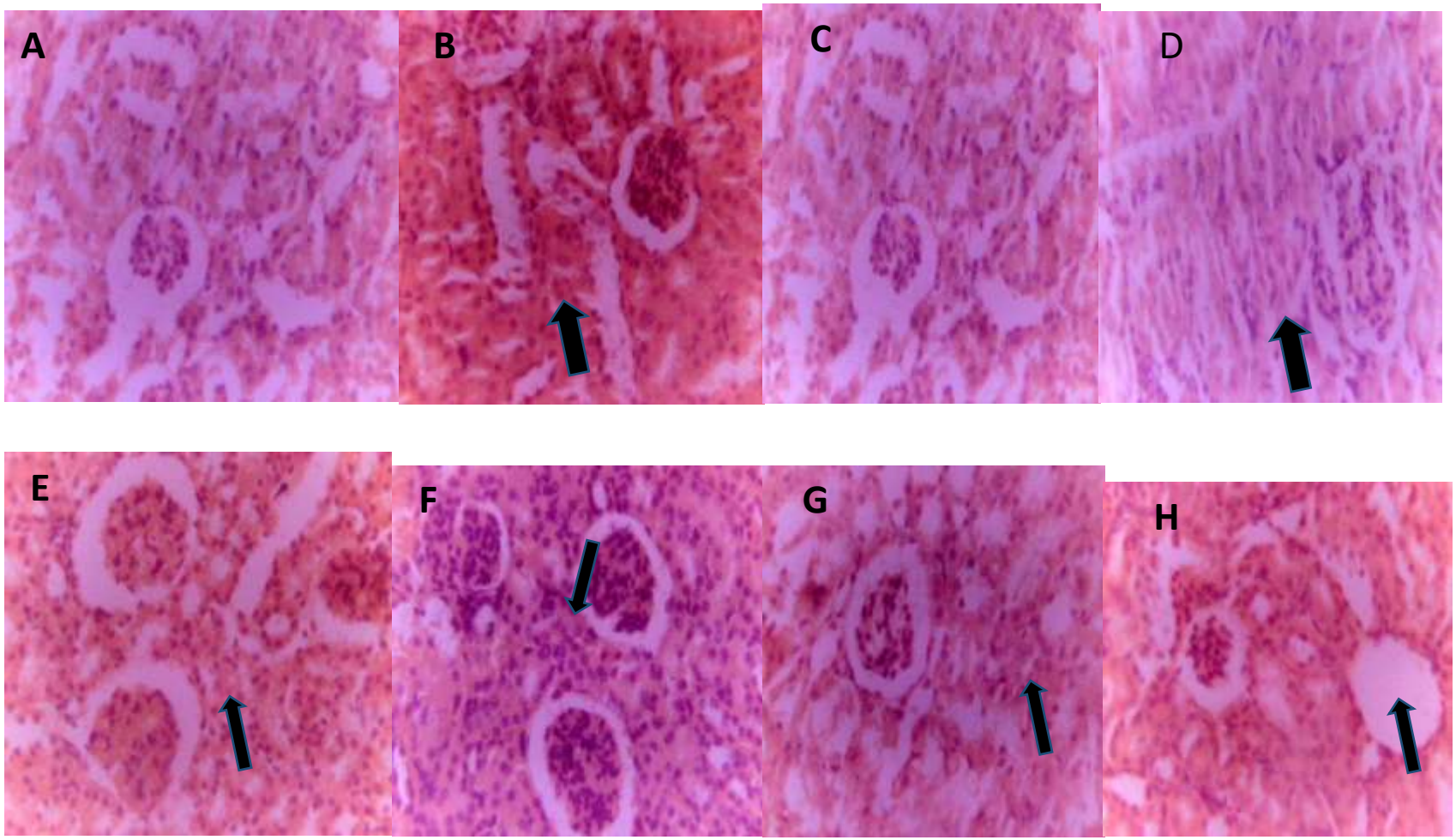

Plate IV: Photomicrograph of Kidney sections of Mice H&E ×400: A: Negative Control showing normal kidney, B: treated with chloroquine (20mg/kg) showing lymphocyte hyperplasia, C: treated with treated with methanol extracts 250mg/kg showing normal kidney, D: treated with methanol extracts 500mg/kg showing tubular necrosis, E: treated with methanol 750mg/kg showing tubular adhesion, F: treated with butanol fraction 250mg/kg showing lymphocyte hyperplasia and tubular adhesion, G: treated with butanol fraction 500mg/kg showing tubular necrosis H: treated with butanol fraction 750mg/kg showing glomerular necrosis

CHAPTER FIVE

5.0 DISCUSSION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Venkatalakshion, 2012). This plant based traditional medicinal system continues to play an essential role in health care, with about 80% of the world` inhabitants relying mainly on traditional medicines for their primary health care (Omonkhelin *et al*., 2007). Plant materials have remained an important source of medicine in the fight against malaria (Aderounmu, 2007) since widely used antimalarial drugs such as quinine and artemisinin are isolated from plants (Rosenthal, 2001).

The *Jatropha curcas* leaf used for this research was dried, ground and stored at low temperatures. This was done to preserve the presence and the quality of the compounds found within the plant. According to Makkar (2000), the moisture content of fresh plant material changes the chemical composition and properties of the plant over time. Grinding of the plant material into fine particles was to create a larger surface area to allow higher extraction of bioactive compounds. The successful extraction of bioactive compounds from plants, according to Parekh *et al*. (2005) is largely dependent on the type of solvent used in the extraction procedure. Effective extraction from the dried plant material was achieved using different solvents. The dried plant material was extracted using water (a similar extraction method employed by traditional healers in preparing most of their herbal remedies) and some organic solvent systems (methanol, ethyl-acetate, butanol and n-hexane) to obtain the bioactive compounds present in the plant under pharmalogical investigation.

Both aqueous and organic extractions were carried out in order to isolate both polar and non-polar compounds. Whilst the pattern for finding the compounds was similar, the abundance varied with methanol showing higher levels of the compounds isolated. This is consistent with other studies which illustrate that plant phytochemicals are more soluble in organic solvents (Willcox *et al*., 2004). These authors further maintains that organic solvent extractions are used as a good alternative in evaluating the antimalarial activities of plants, as organic solvents are able to extract a broad spectrum of chemical constituents. Even though, it is interesting to note that most medicinal plant preparations are conducted in aqueous solvents (water) traditionally, it is noteworthy to observe the effect of organic solvents on the plant material and to describe them.

In this study, the preliminary qualitative phytochemicals screening of the aqueous, methanol, ethyl-acetate and butanol extracts of the *J. curcas* leavesrevealed the presence of tannins, alkaloid, saponins, flavonoid, carbohydrates, terpenoids, glycosides, steroids and cardiac glycosides. While n-hexane fraction only showed the presence of alkaloids, glycosides, cardiac glycosides and steroids.Anthraquinone was absent in all the extracts tested (Table 4.2). The presence of these secondary metabolites is in agreement with work of Sarkiyayi *et al.* (2016) in aqueous extraction of stem bark of *J. curcas.* Also Dharani *et al*. (2008) explained that common antimalarial plants used to treat malaria in traditional medicine contain secondary metabolites, such as alkaloids, terpenoids, coumarins, flavonoids, chalcones, quinines and xanthones.

Alkaloids, terpenoids and tannins detected in *J. curcas* leaveshave been implicated for their antiplasmodial activity in previous study (Sarkiyayi *et al.*, 2016). Quinine, one of the most important and oldest antimalarial drugs, belongs to the class of alkaloids (2003). Table 4.4 revealed that no death caused by an oral dose of 5000 mg/kg body weight of the extracts and its

various fractionscould imply the safety of the plant to be used in the treatment of malaria as also suggested in Sarkiyayi *et al.* (2016).

The results of Gas chromatography mass spectroscopy analysis (Table 4.3) revealed that methanol extracts of *J. curcas* leaves contains eighteen (18) bio active ingredients. Amongst the bio active compounds phytol recorded the highest area percentage of 31.64%. Phytol has been reported to be a very commonly occurring diterpene alcohol in the plant (Saxena *et al.,* 2003). A literature search revealed that phytol was another acyclic terpenoid, occurring as major constituent in several Indian medicinal plants. Reports have shown that phytol possesses antimalarial activity (da Silva *et al*., 2004; Grace *et al*., 2012). The phytol derivative PhY-3 has also been reported to possess significant anti-malarial potential in both in vitro and in vivo bioassay and also non-toxic even at the higher dose to the mice (Saxena *et al.,* 2003).

The acute toxicity result of the present study suggested that the oral medial lethal dose (LD_{50}) of the extract could be greater than 5000 mg/kg body weight as per OECD guideline No 425 (2003). The experimental determination of lack of acute toxicity at the extract dose of up to 5000 mg/kg body weight of mice may be the reason why it is used traditionally in the treatment of malaria and fever.

Analysis of test results indicated significant parasitaemia suppression by all the doses of aqueous and methanol extracts of *J. curcas* and its fractions (table 4.5) as compared to the positive control after the 4-day suppression test. *In vivo* antiplasmodial activity can be classified as moderate, good and very good if an extract displayed a respective percent parasite suppression equal to or greater than 50% at doses of 500, 250 and 100 mg/kg body weight per day (Muñoz *et al.,* 2000;

Deharo*et al.*, 2001). Based on this classification, the crude extracts and its fractions of *J. curcas*are considered to have exhibited good antiplasmodial activity, with dose dependent suppression against *P. berghei*infection in mice.The parasite suppression exhibited by these extracts is comparable to results of former studies conducted on aqueous stem extract of *J.curcas* (Sarkiyayi *et al.*, 2016). The presence of phytochemicals such as flavonoids, terpenoids, phenols, alkaloids, glycosides, tannins, steroids, and saponins in the extract which have earlier been shown to have antiplasmodial effects (Saxena *et al*., 2003; Khaomek *et al*., 2008; Osmund *et al.*, 2019), could be responsible for the antimalarial effect observed in this study. Specifically, the presence of alkaloids in this plant is in accordance with the report of Sarkiyayi *et al*. (2016), who described the main active ingredients in stem bark of *J. curcas* as alkaloids, glycosides, saponin and terpenoids.

During the curative activities as revealed in Fig. 4.2, the extracts also showed significant reduction in level of parasitaemia in extract treated group compared to negative group with methanolic extract displaying highest activity at 750 mg/kg. The crude methanol extract and butanol fractions of *J. curcas* leaves were found to demonstrate higher percentage of parasitaemia suppression as compared to other treatment groups of the plant possibly suggesting the better availability of active ingredients in the methanol extracts.

The anti-plasmodial activities of these plant is not surprising due to the fact that a review conducted by Sabandar *et al*. (2013) had shown that, the genus *Jatropha* (Euphorbiaceae)comprises of about 170 species of woody trees, shrubs, sub shrubs or herbs are used in medicinal folklore to cure various diseases of 80% of the human population in Africa,

Asia and Latin America. Species from this genus have been used to cure stomachache, toothache, swelling and inflammation.

In this study, it was observed that the extract was able to exhibit a considerable effect on hematological Parameters comparable to the standard drug. This could be due to sufficient accumulation of the bioactive compounds in the plant extract. The consideration of haematological indices is due to the fact that most changes during malaria are pronounced in the blood and it's forming system (Yakubu *et al.*, 2007). The plant extract significantly prevent the reduction of haematological parameters compared to the negative control. The ability of *Jatropha curcas* to boost the body's macrophage response, which stimulate the lymphocytic system and increase the production of white blood cells is believed to be a major factor in plantseffectiveness against malaria infection (Saxena *et al*., 2003).

Anaemia is a common problem in malaria,it is a condition characterized by decrease in total cell mass of the blood (Adam *et al.*, 2005) and in this study, was evidenced by a decrease in the levels of circulating RBC and PCV in negative control. Packed cell volume is an indicator of the body's ability to transport oxygen and absorbed nutrients. An increased PCV shows a better transportation capacity of the red blood cells (Isaac *et al*., 2013). Packed cell volume is used to assess anaemia, erythrocytosis, haemodilution, and haemoconcentration (Briggs and Bain, 2017). A decrease in PCV indicates anaemia (Briggs and Bain, 2017). Changes in RBC count are the most typical features of malarial infections and anaemia is the most common complication associated with malaria infection (Erhart *et al*., 2004). The ability of the *J. curcas* to prevent reduction of PCV, RBC and WBC count in parasitized treated mice when compared with

positive control as indicated in table 4.6 and 4.10 suggests that the extract possess hematinic, erythropoietic and immunostimulatory effects.

The lysis of the RBCs may be as a result of non-immune damage of the parasitized RBCs as a result of high parasitemia or immune mediated damage of parasitized and non-parasitized RBCs because the alteration in the RBCs antigen structure caused the parasite invasion to stimulate the production of antibodies against the RBCs (Ugwu *et al.,* 2013). The observed reduction in the PCV, RBC and WBC of positive control group may be as result of repeated haemolysis of the parasitized RBC, Therefore, this triggers immune mediated RBC lysis. Also, the developing parasite destroyed the intracellular protein that is haemoglobin this may accounts for further decrease in the levels of PCV (Ugwu *et al.,* 2013).

Anemia, body weight loss and body temperature reduction are the general features of malaria-infected mice (Langhome*et al.,* 2002). Thus antimalarial agents are expected to prevent body weight loss in infected mice due to rise in parasitaemia (Lukman *et al.,* 2016). The crude extracts (aqueous and methanolic) and fractions (ethyl acetate, butanol and n-hexane) of leaves of *J. curcas*significantly prevented weight loss. Comparable effects in preventing weight loss were also reported in studies conducted on hydro-alcoholic extract of *Asparagus africanus*(Dikasso *et al.,* 2006). A decrease in the metabolic rate of infected mice occurs before death and is accompanied by a corresponding decrease in internal body temperature (Mengiste *et al.,* 2012). All the treated groups demonstrated protective effect against temperature reduction, likely suggesting the presence of constituents in the extracts responsible for such effect (table 4.8 and 4.12).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The following conclusions can be drawn from the study

i. The preliminary qualitative phytochemical screening of the aqueous, methanol, ethyl-acetate and butanol extracts of the *J. curcas* leavesrevealed the presence of tannins, alkaloid, saponins, flavonoid, carbohydrates, terpenoids, glycosides, steroids and cardiac glycosides; seventeen (17) bioactive compounds were identified and the major ones include Phytol (31.64%), Octadecanoic acid (18.30%) and 9,12,15-Octadecatrienoic acid (11.20%).

ii. Aqueous and methanol leaf extracts of *Jatropha curcas* and its fractions had a better suppressive anti-plasmodial activities at 750mg/kg.

iii. Methanolic leaf extracts of *Jatropha curcas* significantly protected the reduction of PCV, RBC and WBC level at a dose of 500mg/kg.

iv. Aqueous and methanol leaf extracts of *Jatropha curcas* and its fractions had ameliorative activities on the histology of intestine, heart, liver and kidney

6.2 Recommendations

i. *Jatropha curcas* leaves extracts is relatively safe and can be utilized as an anti-plasmodial agent

ii. The antiplasmodial activity for other routes of administration should be tested

iii. Further research on aqueous and methanol extract and fractions of *J. curcas* leaves could be carried out in order to isolate, identify and characterize the active compound from this plant

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