EVALUATION OF THE EFFECT OF DETARIUM MICROCARPUM ON LIVER AND SPLEEN OF *P. BERGHEI* INFECTED MICE

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CERIFICATION

We certify that this project was carried out by **ANIEBUE PATRICK TOCHUKWU** with matriculation number **LSC1705062** in the Department of Biochemistry, Faculty of Life Sciences, University of Benin City, Edo State, under our supervision.

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DEDICATION

This work is dedicated to God Almighty for His love, mercies, favor, grace, and strength.

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ABSTRACT

A female mosquito of the species Anopheles that has been infected with the Plasmodium parasites that causes malaria will bite a human victim. Malaria continues to be the biggest cause of death worldwide, although undesirable results can be avoided with early diagnosis and prompt treatment.

Parasites on several vertebrates, some in tissue and others in red blood cells. Five of the 172 Plasmodium species that exist can infect people. *P. malariae, P. falciparum, P. vivax, P. ovale*, and *P. knowlesi* are among them. The zoonotic malaria *P. knowlesi* is known to exist in South-East Asia.

While malaria occurs as an imported disease from endemic places in the industrialized world, it is the most prevalent disease in Africa and several countries of Asia. The fight to eradicate malaria began on a global scale in 1955. A global malaria control program is run by the World Health Organization, with an emphasis on strengthening primary healthcare locally, early disease detection, prompt treatment, and disease prevention.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Malaria is a tropical disease caused by blood parasites such as *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium vivax*. Malaria is primarily caused in Nigeria by *P. falciparum* and *P. malariae*. These parasites are transmitted to humans by the female anopheles mosquito. Malaria has the highest morbidity and mortality rates of any infectious disease in the world (World Malarial Report, 2005; Smith, 1978; WHO, 2000). According to a survey, Sub-Saharan Africa accounts for 90% of all malaria cases worldwide. This region is home to nine out of ten cases of this disease, which claims over one million lives each year (World Malarial Report, 2005; Africa Union Memoir, 2005). The symptoms of "uncomplicated" malaria commonly include headache, malaise, weariness, body aches, nausea, and vomiting in addition to a string of recurrent episodes of chills, a high temperature, and sweating. The condition can evolve to "severe malaria" in some instances, particularly in populations like children and expectant women, and include complications like cerebral malaria/coma, seizures, severe anemia, respiratory distress, kidney and liver failure, circulatory collapse, and shock.

Most anti-malarial medications used, which mostly consist of chloroquine, mefloquine, quinine, quinidine, doxycycline, clindamycin, and artemisinins, are effective against blood parasite stages (the kinds that produce symptoms). Anti-malarial medications are often utilized, but their therapeutic efficacy is constrained by the escalating development of parasite resistance. For instance, in several of the malaria endemic locations, chloroquine, the preferred medication most frequently used to treat malaria in Africa, is no longer effective. In many parts of the world, artemisinin-based combination therapy is currently advised as a treatment for falciparum malaria. The development of treatment resistance in malaria parasites, however, is a grave issue.

Chloroquine is used for both treatment and prophylaxis. Its prolonged dosage may result in cardiac block and myopathy that worsens (Verlinden BK and Louw A, 2016). Additionally, it has been linked to toxicity, mainly in the retina (Regillo CD 2011). Rheumatoid arthritis and systemic lupus erythematosus are two autoimmune diseases that are frequently treated with

chloroquine. Chloroquine disrupts the lysosomal breakdown of proteins by raising the pH in lysosomal lumens (Tonnesmann E *et al.,* 2013)

In locations where chloroquine resistance exists, quinine is often used, although it can have major toxic consequences, including arrhythmia, angina, hypotension, circulatory defects, and shock. There have been published case reports of patients with severe malaria who developed an arrhythmia while receiving quinine treatment (Gunawan CA and Harijanto PN 2007).

Artesunate has been used as an option to treat malaria with little side effects. However, it has been demonstrated to have an impact on the smooth muscles of the 201 airways, preventing human cultured smooth muscle cells from proliferating and resulting in hyperplasia and hypertrophy (Tan SS *et al.,* 2014) which obstructs the airways (Lambert RK *et al.,* 1993)

The development of innovative medications frequently begins with ethnomedicinal plants (Nogueira HM and Gama RD 2009). A range of regional management strategies were influenced by the plant's excellent quality firewood and traditional pharmacological uses for its roots, leaves, and bark. Traditional medicine regularly used the *Detarium microcarpum* to cure a wide range of conditions, including diarrhea, bronchitis, fever, meningitis, convulsions, malaria, diabetes, bacterial, and fungal infections (Mishra SK *et al.,* 2007). Leguminosae *D. microcarpum* is one of the most significant medicinal plants in Africa. The Kilba tribe of Northeast Nigeria has reportedly utilized the stem and root barks as a treatment for newborn illnesses such "Gedigedi" or "Tando" (Hausa), stomach problems, wounds, and other conditions. Eaten as a vegetable and condiment, the seeds and leaves. The fruit pulp is used to treat skin diseases in Burkina Faso. The bark, leaves, and roots of *D. microcarpum* have also been used medicinally as a diuretic and astringent (Bouyate and Sam, 2006). This plant's various sections have been linked to antiplasmodial and antirheumatic properties (Abreu *et al.,* 2014)

1.2 Objectivity of Study

The following objectives were pursued to fulfill this aim.

1. To look into the antiplasmodial activity of *D.microcapum* stem bark as a potential antimalarial treatment.

2. To access the cytotoxic effects of methanol stem bark extract on mice infected with *P. beghei*

3. To determine the effect on some oxidative stress parameters like MDA, LDH

1.3 Literature Review

Malaria, which continues to be among the most dangerous, life-threatening infectious diseases, has had a significant impact on human lives for thousands of years (Miller *et al.,* 2013; White *et al.,* 2014; Cowman *et al.,* 2016). The protozoal disease known as malaria, which is spread by mosquitoes, predates written history. Man, and malaria are thought to have coevolved, and populations of human malaria may have originated in West Africa (Rugemalila *et al.,* 2006). The most significant insect-transmitted disease is by far malaria. According to the most recent estimates from the World Health Organization, there are 300–500 million cases of clinical malaria year, and 1.4–2.6 million people die from it, many of them African children. As a result, malaria is the sole major preventable cause of newborn death. In Nigeria, malaria accounts for up to 60% of outpatient visits to healthcare facilities and 30% of all hospital admissions. In addition to an estimated 300,000 annual deaths, malaria is thought to be the cause of roughly 110 million clinical cases, 25% newborn mortality, 30% childhood mortality, and 11% maternal mortality. According to estimates, the annual cost of this disease in Nigeria in terms of medical expenses, preventative measures, missed labor hours, etc. is N132 billion (FMOH, 2005b; 2009d).

1.4 Aim of Study

The aim of this study is to evaluate the vivo antiplasmodial activity of methanol fraction of *D.microcarpum* stem bark extract on some liver function enzymes, and oxidative and biochemical changes induced by *P. beghei* infected mice

1.5 Discovery of Malaria

It is thought that malaria outbreaks have existed since the dawn of humanity. It is the most prevalent sickness that has caused numerous fatalities and is even suspected of being to blame for major military losses and the demise of several nations (Lehrer, 1981). Ancient Chinese medical records from 2700 BC and the Ebers Papyrus, written 1200 years later,

provide the earliest descriptions of malaria (White *et al.,* 2014). Alexander the Great, a military genius, passed away from malaria (Lehrer, 1981). The fact that Cesare Borgia, Christopher Columbus, Albrecht Dürer, and George Washington were all affected by this condition shows that it was prevalent throughout all societal strata (Dugacki, 2005; Moss *et al.,* 2008)

1.6 Clinical Symptoms of Malaria

The fever pattern, which is the initial sign of the illness, is similar to the early stages of many other bacterial, viral, and parasite infections. *P. vivax, P. malariae*, and *P. ovale* are the first three species, and while they can be very sickening, they are not usually fatal. But the fourth specie (*P. falciparum*), which can occasionally result in coma and death within a few days of infection, creates a considerably more serious and progressive sickness (Okell *et al.,* 2012). Numerous names have been given to this parasite, including cerebral malaria, malignant tertian malaria, and autumnal fever. Typically, the incubation phase lasts 7 to 14 days. The most severe form of malaria infection includes a temperature (which can exceed 40Co in non-immune patients), chills, sweats, cough, diarrhea, respiratory distress, headache, shock, renal and hepatic failure, pulmonary and cerebral edema, coma, and death. Over 10% of cases involving untreated youngsters and non-immune adults result in death (Nkogbe and Akue, 2011).

1.7 Epidemiology

1.7.1 The Vector

The parasites that cause human malaria are only spread by about 40 species of the mosquito genus Anopheles (Bhatt *et al.,* 2015). High levels of the steroid hormone 20-hydroxyecdysone are transferred to female Anopheles spp. During mating, and the presence of this hormone has been linked to favorable Plasmodium spp. (Mitchell *et al.,* 2015)

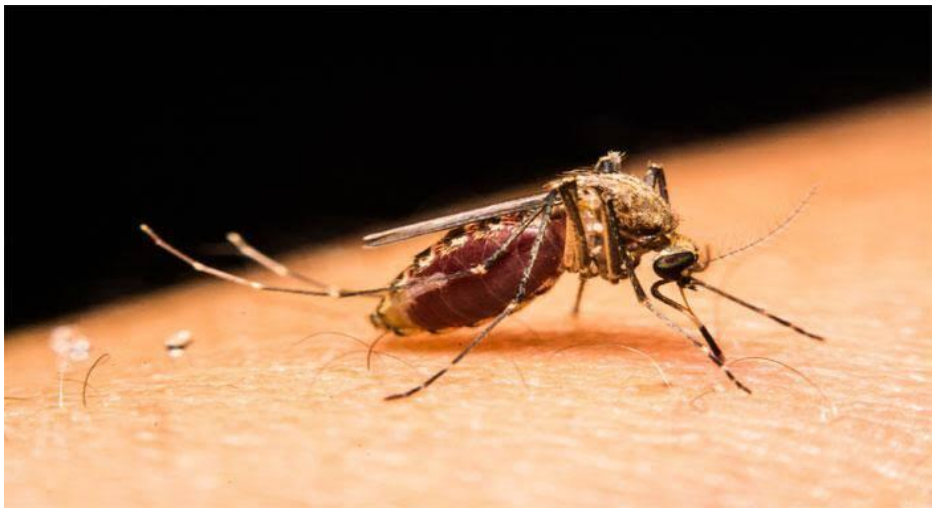


Plate 1: Female Anopheles Mosquito

1.7.2 The Parasite

Plasmodium spp. are single-celled eukaryotic organisms that are a part of the phylum Apicomplexa, which is named for the apical complex that is involved in host cell invasion. (Florens *et al.,* 2002, Gardner *et al.,* 2002). A microscopic protozoon from the Plasmodium species family, which includes multiple subspecies, is the cause of malaria. The parasites that cause malaria in humans come in four different species*: Plasmodium malariae , P. vivax , P. falciparum* (Welch), and *P. ovale*

1.7.3 The Disease

People living in resource-constrained regions of Africa, Asia, and Central and South America continue to bear a heavy burden from malaria. In 2015, there were reportedly 214 million cases of malaria (WHO, 2015). With 88% of the cases, Africa in carries the heaviest burden. Southeast Asia is second with 10%, followed by the eastern Mediterranean with 2%, and Central and South America with 1%. Because they have lost their maternal antibodies but have not yet formed a protective antibody, children older than 6 months are more vulnerable. Due to frequent exposure to the parasite, adults, and children older than five years old who reside in areas where P. falciparum is transmitted year-round develop a partial protective immunity. There is proof that immunity to P. vivax develops more quickly (Miller *et al.,* 2013)

1.7.4 The Plasmodium spp. Life Cycle

During a blood meal, the mosquito vector transmits the Plasmodium spp. parasite to the host in the sporozoite stage. Sporozoites enter liver cells within 30 to 60 minutes, when they multiply and divide into merozoites. Merozoites are released into the bloodstream when an infected liver cell bursts, starting the disease's symptomatic asexual reproductive stage in which, they invade red blood cells. Four to eight days after the initial red blood cell invasion, symptoms appear. Within the red blood cells, the merozoites replicate for 36–72 hours (from red blood cell invasion to hemolysis).

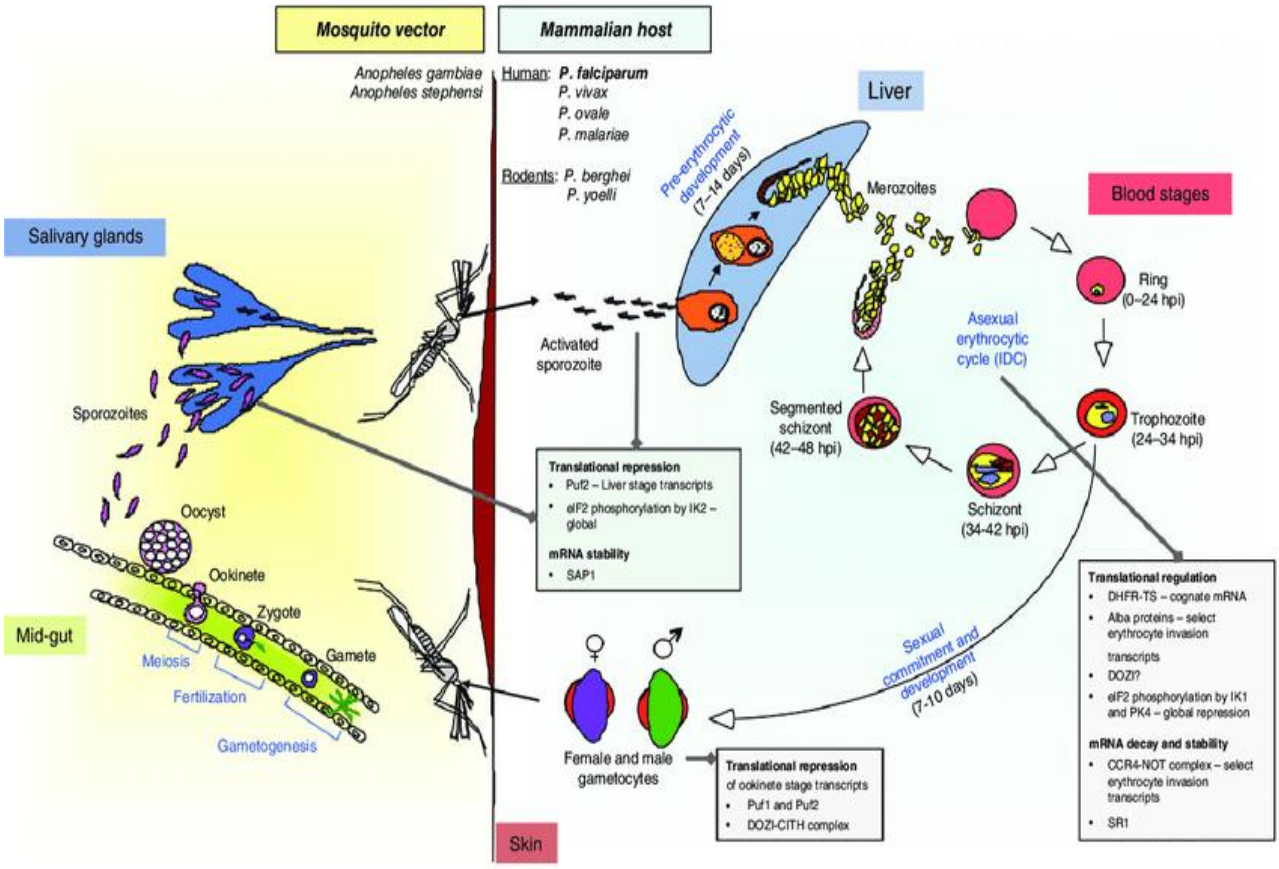


Plate 2: The Plasmodium spp. life cycle.
Source: Howling and Lina, 2015.

Additionally, the hypnozoite stage of Plasmodium vivax and *Plasmodium ovale* can be reached in the liver. Red blood cells can release merozoites that can infiltrate other red blood cells, multiply, or, under rare circumstances, differentiate into male or female gametocytes (Baker, 2010; Waters, 2016). It has been demonstrated that the transcription factor AP2-G controls the decision to commit to gametocytogenesis. The mosquito vector consumes the concentrated gametocytes in the skin capillaries during a subsequent blood meal. Each male gametocyte undergoes three rounds of mitosis in the mosquito's gut, producing eight microgametes; the female gametocyte develops into a macrogamete. The male and female gametocytes unite to generate a diploid zygote, which enlarges into an ookinete and leaves the gut through the epithelium as an oocyst (Annan *et al.,* 2007). Oocysts go through replication cycles and develop into sporozoites, which go from the mosquito's abdomen to the salivary glands. As a result, 7–10 days after feeding on blood containing gametocytes, the mosquito may be "armed" and capable of transmitting Plasmodium spp. to another person through a bite.

1.8 Mechanisms

1.8.1 The Red Blood Cell Stage

Since red blood cells are the site of a lot of parasite reproduction, the red blood cell stage of Plasmodium spp. infection is what causes symptoms of malaria.

1.8.2 Invasion

Plasmodium spp. parasites enter the host erythrocyte (mature red blood cell) or reticulocyte (immature red blood cell) through specific ligand-receptor interactions that are mediated by proteins on the surface of the parasite (Paul *et al.,* 2015). P. vivax and other species primarily infect reticulocytes, which are less common than erythrocytes, whereas P. falciparum can invade and multiply in both reticulocytes and erythrocytes (Lim *et al.,* 2016). However, for P. falciparum, two essential red blood cell receptors (basigin and complement decay-accelerating factor, also known as CD55) have been identified. Most parasite erythrocyte-binding proteins or reticulocyte-binding proteins that have been linked to invasion are redundant or are expressed as a family of variant forms.

1.8.3 Replication

Once inside the red blood cell, Plasmodium spp. release hundreds of proteins that affect pathogenesis, cell adhesion and sequestration in tissues, and nutrient uptake in the host cell (Boddey and Cowman, 2013; Spillman *et al.,* 2015; Cowman *et al.,* 2016).

Plasmodium species proliferate quickly in red blood cells, and during symptomatic disease, the parasites may multiply exponentially to >1012 parasites per patient. Numerous antimalarial target pyrimidine biosynthesis because this fast expansion necessitates persistent pools of nucleotides for the synthesis of DNA and RNA (Phillips, 2011). All the necessary amino acids are provided by auxotrophic Plasmodium species (that is, they must acquire all of these from food because they cannot synthesize them from precursors).

1.8.4 Immune Evasion and Host Immunity

In one study (Rosenberg et al., 1990), the number of sporozoites that are injected into the skin was estimated to be around 15. These sporozoites may be phagocytosed by dendritic cells for antigen presentation in the lymph node draining the skin inoculation site (Sinnis and Zavala, 2012). The number of sporozoites that can concurrently travel through the mosquito's proximal duct is limited by the duct diameter, but the likelihood of transmission is raised when the host is bitten by mosquitoes that carry a greater number of sporozoites (Frischknecht *et al.,* 2004). It is unclear how some sporozoites may enter the liver and infect the hepatocytes while interacting with numerous other immune system effectors. The ability of sporozoites to inhibit the activity of Kupffer cells, also known as stellate macrophages, which are the resident macrophages of the liver, and to regulate the expression of genes that encode MHC class I molecules may help to explain immune evasion in the liver (Tang *et al.,* 2014).

1.8.5 Pathogenesis

The predominant pathogenic mechanism is the haemolysis of Plasmodium spp.-infected red blood cells, which release parasites and malaria endotoxin ― understood to be a complex of haemozoin and parasite DNA, which trigger Toll-like receptor 9 (TLR9), a nucleotide-sensing receptor involved in the host immune response against pathogens (Parroche *et al.,* 2007) ― that leads to high levels of tumour necrosis factor (TNF) production and to clinical

symptoms such as fever (Karunaweera *et al*., 1992; Wijesekera *et al*., 1996; Vijaykumar *et al*., 2001).

1.8.6 Parasite Factors That Influence Disease Severity

Surface proteins produced by the parasite are associated with disease severity and pathogenesis. The var gene family, which has about 60 members, encodes a significant surface antigen in *P. falciparum* (Smith, 2014; Wassmer *et al.,* 2015; Duraisingh *et al.,* 2016). Based on their genomic location and sequence, the majority of the var genes are divided into three subfamilies: A, B, and C. The B and C groups mediate binding to host cells via CD36, also known as platelet glycoprotein (Baker, 2010), whereas the A group genes mediate non-CD36 binding interactions that have been linked to severe malaria, including cerebral malaria (Smith, 2014; Duraisingh *et al.,* 2016).

1.9 Host Traits That Influence Disease Severity

Strong selection pressure from malaria has influenced the development of the human genome (Lopez *et al.,* 2010; Piel, 2016). In populations residing in malaria-endemic areas, some haemoglobin-encoding alleles that in homozygous genotypes result in severe blood disorders (such as thalassaemia, the earliest described example, and sickle cell disease) have been positively selected because heterozygous genotypes defend against malaria (Elguero *et al.,* 2015). Malaria can also be prevented by other hereditary hemoglobin defects, such as mutations affecting hemoglobin C and hemoglobin E (Kwiatkowski, 2005). Additionally, genetic variants that influence the red blood cell-expressed proteins or that cause enzyme shortages can potentially offer protection against serious illness. A crucial receptor for P. vivax invasion, the red blood cell Duffy antigen interacts with the Duffy antigen-binding protein on the parasite surface (Paul *et al.,* 2015). Although the discovery of Duffy antigen-negative individuals who can contract P. vivax suggests that we still have a limited understanding of the factors involved in P. vivax invasion, the genetic inheritance of mutations in ACKR1 (which encodes the Duffy antigen) in Africa is credited with slowing the spread of P. vivax in this continent (Cheng *et al.,* 2016; Gunalan *et al.,* 2016).

1.9.1 Diagnosis, Screening and Prevention Diagnosis

Fever and the presence of parasites are two important disease pathology factors considered by the WHO when diagnosing malaria (WHO, 2016). Rapid diagnostic tests (RDTs) or light microscopic inspection of a blood smear can both be used to identify parasites (WHO, 2016). The diagnosis may be aided by the patient's exposure risk (for instance, whether the patient resides in an area where the disease is endemic or their travel history). Additionally, the degree of transmission of the species in the region is correlated with the clinical manifestation of Plasmodium spp. infection. Consistent high fever bouts are one of the uncomplicated malaria symptoms; when high parasitemia levels are reached, various potentially fatal consequences may happen (severe malaria) The majority of severe malarial complications are caused by infected red blood cells blocking blood vessels; the severity and symptoms of these complications vary by age and depend on which organs are affected and to what extent. Lung and kidney disease are uncommon in African children but frequent in non-immune adults.

1.9.2 Parasitemia

Parasitemia in patients with uncomplicated malaria is normally between 1,000 and 50,000 parasites per microliter of blood. There is no cut-off density and the association between the greater numbers and severe malaria is shaky. The thick-smear microscopy detection threshold is 50 parasites per microliter (Joanny *et al.,* 2014). Since parasitemia can rise 20-fold in a 48-hour cycle, the capacity to identify low levels of parasitemia is crucial for anticipating clinical relapses.

1.9.3 Rapid Diagnostic Test (RDTs)

RDTs have sensitivities comparable to those of light microscopy examinations, are based on the immunological detection of parasite antigens (such as lactate dehydrogenase (LDH) or histidine-rich protein) in the blood and have the advantage of not requiring substantial user training. These assays provide quick diagnosis at the point of care in environments with limited resources, which can significantly enhance malaria control. Misleading-positive RDT results can occasionally be troublesome, though, as they may provide the false impression that antimalarial medications are ineffective. According to reports, deletions of the pfhrp2 gene in South American P. falciparum strains have led to false-negative test findings

(Gamboa *et al.,* 2010; Akinyi *et al.,* 2013; Cheng et al., 2014; Deme *et al.,* 2014; Bharti *et al.,* 2016; Parr *et al.,* 2016).

1.10 Prevention of Malarial in Vulnerable Populations

Vaccines, chemoprevention, and vector control are all effective ways to prevent Plasmodium spp. infection.

1.10.1 Vector Control

From the most general to the most specific, mosquito (vector) control techniques include the following: the widespread application of insecticides, such as DDT campaigns; the use of larvicides; the destruction of breeding grounds (draining marshes and other breeding reservoirs); indoor residual insecticide spraying (applying residual insecticide inside homes, on walls, curtains, or other surfaces); and the use of insecticide-treated bed nets. It has also been suggested to utilize endectocides, which are medications like ivermectin that kill or shorten the lifespan of mosquitoes that feed on people who have taken them (Ouédraogo *et al.,* 2015). The degree of safety data needed for the licensing of endectocides for this purpose will need to be high because this strategy is still experimental, and people would be taking medications that have no direct benefit to themselves (as they do not directly prevent human sickness).

1.10.2 Chemoprotection and Chemoprevention

Chemoprotection is the term for the use of drugs (given at prophylactic levels) to temporarily protect individuals entering an area of high endemicity. Seasonal malaria chemoprevention campaigns, intermittent preventative care for children and pregnant women, and mass drug administration have all employed chemoprevention as an effective technique to lower the prevalence of malaria (WHO, 2015). Given that they are administered to many healthy individuals, such antimalarial must have an excellent safety profile.

1.10.3 Vaccines

Effective malaria vaccines are difficult to create because protist pathogens (such Plasmodium spp.) are large-genome microorganisms that have evolved highly effective immune evasion techniques, in contrast to viruses and bacteria, against which effective vaccines have been

established (such as encoding dozens or hundreds of cell surface protein variants). However, the enhanced adjuvants and biotechnology arsenal for producing antigens may help to resolve these problems.

1.11 Management

No single medication is effective against every Plasmodium species or every illness symptom that can appear in various patient populations. Consequently, each situation's treatment needs to be suitably adapted (WHO, 2015; Daily, 2017). First, there are differences between the treatments for simple malaria and severe malaria. The preferred course of treatment for uncomplicated malaria is an oral medication with good efficacy and few side effects. Different medications are utilized for various Plasmodium species, and rather than actual species differences, the selection is typically influenced more by drug resistance rates (which are lower in *P. vivax, P. ovale, P. malariae, and P. knowlesi* than in *P. falciparum*). Thus, chloroquine is used in most non-P. falciparum malaria cases where it is still effective due to its low cost and excellent safety, whereas P. falciparum malaria requires newer drugs that overcome resistance issues. Additional therapies are required due to the survival of *P. vivax* and *P. ovale* hypnozoites even after the stages that cause symptoms have been eliminated. Primate only affects hypnozoites.

1.11.1 P. falciparum Malaria

Fixed-dose combinations of two medications, ACTs, are the mainstay therapies for uncomplicated *P. falciparum* malaria (WHO, 2015). Quinine has been used in medicine for centuries (Harrison, 2015), but it wasn't until the middle of the 20th century that a synthetic form was created, and it was then that the burgeoning pharmaceutical and government research sectors brought forth the subsequent generation of drugs that were based on it. The preferred combination partners are 4-aminoquinolines (such as amodiaquine, piperaquine, and pyronaridine) and amino-alcohols (such as lumefantrine or mefloquine), which are thought to prevent the production of hemozymes.

1.11.2 P. vivax Malaria

WHO recommends chloroquine or ACTs for treating *P. vivax* malaria that isn't difficult (WHO, 2015) Although only artesunate-pyronaridine is approved for the treatment of blood-

stage P. vivax malaria, the other ACTs are as effective and are used off-label as chloroquine-resistant P. vivax becomes more prevalent, particularly in Asia. Malaria control is hampered by relapses of P. vivax malaria. Phase III clinical investigations for the next-generation 8-aminoquinoline tafenoquine (Llanos-Cuentas *et al.,* 2014) are currently being conducted. To guarantee safe

usage of the medication and establish the ideal dose, patients taking tafenoquine will still need to have their G6PD enzyme activity evaluated, much like patients getting primaquine.

1.11.3 Drug Resistance

The pharmacokinetic characteristics of the two medications in ACTs in patients vary greatly. Even though the plasma half-life of the artemisinin components is only a few hours, they can reduce parasitemia by three to four orders of magnitude. The terminal half-lives of 4-aminoquinolines and amino-alcohols, in contrast, are long (>4 days), resulting in cure (defined as an acceptable clinical and parasitological response) and different degrees of post-treatment prophylaxis.

However, the efficiency of the ACTs in quickly reducing parasitemia suggests that any emerging resistance has primarily developed as a result of subpar clinical practice, such as the use of artemisinin derivatives as monotherapy, a lack of patient compliance, and low-quality medicines (including fake drugs); these are all circumstances in which numerous parasites are exposed to a single active molecule (White, 2017).

1.12 Medicinal plants

The development of Plasmodium strains that are resistant to currently available medications, particularly P. falciparum, has presented a significant problem in the treatment and prevention of malaria (Cui *et al.,* 2015). Due to the lack of inexpensive access to conventional treatments, almost 70% of Africans rely on traditional medicine (Murithi *et al.,* 2014). Since there are few and understaffed rural health institutions in Nigeria, individuals often turn to traditional herbal remedies to cure malaria. The oldest and most well-known method of treating illness still involves the use of herbs (Chikezie *et al.,* 2015). Scientists are currently interested in the utilization of medicinal plants in Africa to support the ethno-medical uses as a treatment for specific disorders.

When herbal remedies are demonstrated to be effective and safe, the World Health Organization (WHO) recommends their use (Falodun *et al.,* 2009). These plants' therapeutic usefulness comes from certain chemical compounds (secondary metabolites) that have a clear physiological effect on the human body (Edeoga *et al.,* 2005). Plant active substances, commonly referred to as phytochemicals or bioactive compounds from plants, are substances that are produced naturally and have a variety of biological effects on both humans and plants. These plant compounds are produced primarily to shield the plants from pests and illnesses (Akinmoladun *et al.,* 2007; Airaodion *et al.,* 2009).

Since medicinal plants contain several metabolites with a wide range of structural and pharmacological properties, they are a potential source of novel antimalarial medications (Ntie-Kang *et al.,* 2014). The majority of medicinal plants with secondary metabolites in their crude extracts and solvent fractions have been shown to have antiplasmodial effects in prior investigations (Bankole *et al.,* 2016). Examples include the chloroform extract of Croton macrostachyus (Bantie et al., 2014), the aqueous leaf extracts of Markhamia tomentosa and Polyalthia longifolia (Bankole *et al.,* 2016), the N-butanol fraction of Dodonaea angustifolia (Amelo *et al.,* 2014), and the Osiris quadripartite (Girma Some of the 40 species of Nuxia (Al-Massarani *et al.,* 2017) are used in Madagascar to cure malaria (Neergheen-Bhujun, 2013).

1.12.1 Detarium

Africa is the natural home of the genus Detarium (Fabiaceae, Subfamily Caesalpiniaceae). It is a member of the Detarieae tribe. There are three significant species presents. Detarium senegalense J.F. Gmelin, D. macrocarpum Harms, and *D. microcarpum* Guill

1.12.2 Detarium Microcarpum Guill

D. microcarpum Guill has been utilized for decades to treat a variety of ailments in numerous societies throughout Africa. African tree *D. microcarpum* Guill is a member of the Fabaceae family (legumes). Due to its medicinal characteristics, edible fruit (which can be consumed raw, boiled, or turned into flour with a variety of purposes of its own), and hardwood used as fuelwood, it is a versatile species with a wide range of uses.

1.12.3 Scientific Classification

Taxonomic profile of Detarium microcarpum

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Subfamily: Caesalpinioideae

Genius: Detarium

Species: Microcarpum Guill

1.12.4 Botanical Description

D. microcarpum is a small tree or shrub with a horizontal root system that can grow up to 10-15 meters tall but can reach 25 meters in moist areas. It only exists in the arid parts of central and west Africa. Usually, it belongs to the dry savanna species (Leung *et al.,* 1968). Compared to seedlings, which typically develop to 0.6 m after 3 years and may reach 1.5 m in 4 years, the shoots of the trunk grow much more quickly, reaching a height of 1.5 m to 2 m in 1 to 2 years (Abdalbasit *et al.,* 2009).

Plate 3: *D. microcarpum* leaves
Source: Odoh and Ene, 2020.

1.12.5 Local Names

There are numerous colloquial names for this plant, including sweet dattock or tallow tree in English, dankh or petit détar in French, as well as Abu-laili (Sudan). The plant known as "Ofo" is thought to be a "religious" tree that grows in God's own compound and represents honesty and sincerity among the Ibo tribe of south-eastern Nigeria (Ejizu, 1986). Due to its widespread usage in African traditional medicine, it is the specie of the genus that has been the subject of the most research.

1.12.6 Location and Growing Conditions

The dry parts of West and Central Africa (Benin, Cameroon, Central African Republic, Chad, Gambia, Ghana, Guinea, Guinea Bissau, Côte d'Ivoire, Mali, Niger, Nigeria, Senegal, Sudan, and Togo) are where *D. microcarpum* naturally exists. *D. microcarpum* grows in dry savanna, in contrast to the other species in its family. This species can be propagated vegetatively or from seeds. It is capable of vegetative multiplication through coppice regeneration, suckering

from stumps or roots, and grafting utilizing scions from mature trees, in addition to vegetative propagation through rooted cuttings. This species is primarily found in areas with annual rainfall between 600 and 1000 mm on shallow, rocky, and lateritic soils, frequently on slopes (Kouyate, 2005). It is mainly prevalent in semi-cleared, dry savannahs or wooded savannahs.

1.12.7 Traditional Uses

The Specie *D. microcarpum* is reputed to have medicinal and magical properties throughout western Africa. It is used to cure a variety of illnesses in African ethnomedicine, including syphilis, dysentery, diarrhea, bronchitis, pneumonia, sore throat, malaria, leprosy, and meningitis (Dalziel 1937, Abreu *et al.,* 1995, 1999, Keay *et al.,* 1998, Burkell 1995). The fruit pulp of *D. microcarpum* is used to treat skin infections in Burkina Faso. In Mali, the decoction of the leaves or roots is used to treat paralysis, meningitis, fatigue, cramps, and difficult deliveries. The bark is used to treat measles, itching, hypertension, nocturia, and nocturnal urination (Kouyate 2005). The fruit preparation is used to cure vertigo in Niger and Togo, while a leaf decoction is used to treat convulsions and fainting in Benin.

1.12.8 Phytochemistry

The abundance of numerous bioactive constituents in *D. microcarpum* has been discovered through phytochemical screening of the various crude extracts of the plant. There have been claims that certain of these compounds, particularly flavonoids, tannins, cardiac glycosides, and terpenoids, have antibacterial and therapeutic characteristics (Hassan *et al.,* 2004; Nweze et al., 2004; Nwaogo *et al.,* 2007). According to reports, antinutrients such saponins, phytates, and cyanides are present. The leaves of *D. microcarpum* have also yielded glycosides and alkaloids (Iwu, 1989; Anhwange *et al.,* 2004). The edible fruit is high in vitamin C. (Dalziel, 1995). The *D. microcarpum* seed, which is used to thicken soup traditionally, is high in carbohydrates (70.38%), crude lipid (7.41%), proteins (12.19%), crude fiber (2.63%), ash (3.51%), and moisture (4.3%)

1.13 Pharmacological Activities

The following are a few of the recorded activities:

1.13.1 Antidiabetic Activity

The abundantly present flavonoids in the methanol extract of *D. microcarpum* roots and its fraction dramatically lowered blood sugar levels in alloxan-diabetic rats without causing hypoglycemia (Okolo *et al.,* 2012)

1.13.2 Antibacterial and Antifungal Activities

It has been demonstrated that the ethanol extract of *D. microcarpum* bark has antimicrobial properties against a number of pathogenic organisms, such as Pseudomonas aeruginosa, Klebsiella pneumonia, Citrobacter freunditis, Staphylococcus aureus, Streptococcus pyrogenes, and Listeria monocytogenes. (Abreu and colleagues, 1998) A broad spectrum of antimicrobial activity was seen against clinical isolates of Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella paratyphi, and Candida albicans in a study using the aqueous methanol extract of the seeds of *D. microcarpum* (Ebi and Afieroho, 2011). The presence of steroidal saponins and flavonoids, according to the scientists, is what causes this activity.

1.13.3 Antiviral Activity

It was demonstrated that the flavanes in the methanol extract of *D. microcarpum* significantly inhibited the HIV-1 or HIV-2 virus (Mohmood *et al.* 1993). Additionally, Lymnaea natalensis was significantly molluscicidal to the bark extract of *D. microcarpum* (Mahmood *et al.,* 1993).

1.13.4 Enzyme Inhibition

The clerodane diterpenes isolated from the fruits of *D. microcarpum* (Cavin *et al.,* 2006) was shown to inhibit the enzyme acetylcholinesterase (AChE). One of the compounds-5α,8α (2-oxokolavenic acid was ten times as potent as galanthamine, a clinically useful drug for Alzheimer's disease. Inhibition of AChE is currently the most efficient approach in managing the symptoms of Alzheimer's disease.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 CHEMICALS/REAGENTS

2.1.2 APPARATUS

The following apparatus were used for the study:

- Glass Slides
- Microscope
- EDTA Containers
- Micro-pipette
- Plain Containers
- Universal Containers
- Test-tube racks
- Beakers
- Foil Paper
- Cotton Wool
- Funnel
- Refrigerator
- Cavette
- Test tubes
- Stopwatch
- Syringes
- pH meter
- Glass rod stirrers
- Spatula
- Centrifuge tube
- Filter paper

2.2 PLANT COLLECTION AND AUTHENTICATIONS

In Uwasota, Benin City, Edo State, Nigeria, a private farm provided the *D. microcarpum* leaves. The University of Benin's Department of Pharmacognosy carried out the identification and authentication.

2.2.1 PREPARATION OF PLANT EXTRACT

Fresh leaves of *D. microcarpum* were air-dried in the laboratory. The dried pieces were then crushed into powder by hand. 100g of dried powdered form of plant material was extracted by soaking in 100ml of Methanol for 72 hours (3 days). Stirring was done twice daily and supernatant was obtained by filtering with Muslim clothe at the end of three days. The extract was concentrated in rotary evaporator and final concentration and kept in a refrigerator at 40oC protect from light and moisture for subsequent use.

2.2.2 FRACTIONATION OF THE PLANT EXTRACT

The plant extract dissolved in 250ml of methanol mixture. Then transferred into a clean separating funnel, properly mixed and allowed to stand. 250ml of hexane was added to the separating funnel properly mixed and left for 4hours. The mixture separates into layers, the top layer being composed of n-hexane, and the bottom layer being composed of polar. The addition process proceeded until no appreciable amount of extract appeared to transfer into the n-hexane portion after adding n-hexane and shaking. To obtain dichloromethane, ethyl acetate, and methanol fractions, similar processes were used with each substance.

2.3 EXPERIMENTAL ANIMALS

The infected male mice weighing between 15.0-28.03g were obtained from a private rearing center and were used for the experiments. The mice were housed under standard laboratory conditions at temperature 27+-2^{o}C, relative humidity 70% and at 12 hours day/night cycles. They were properly feed and given water. The animals were allowed to acclimatize for one week before commencing the experiment. The experiments were conducted in strict compliance with internationally accepted principles for laboratory animals use care in the Canadian Council on Animal Care Guidelines and Protocol Review

2.4 INNOCULATION OF ANIMALS

Mice infected with *P. berghei* Nk65 chloroquine strain were obtained from Nigerian Institute of Medical Research (NIMR), Lagos State, Nigeria and kept at the animal house in the Department of Biochemistry, University of Benin, Edo State, Nigeria. 1ml of Parasitized erythrocytes were obtained from donor-infected mice by cardiac puncture and made up to 10ml containing about 10^{14} infected erythrocytes.

2.5 ANTIPLASMODIAL STUDIES (SUPRESSIVE TEST)

Animals were divided into six groups according to their weight; mice with similar weight were placed together. The six groups were divided into three test groups and three control groups

1) Normal control: nPnT
2 Negative control: PnT +0.2ml of Carboxyl methyl cellulose
3) Positive control group: PnT + Chloroquine (10mg/kg)
4) Fraction 1 (Methanol): PnT + 100mg/kg of plant extracts
5) Fraction 2 (Methanol): PnT + 250mg/kg of plant extract
6) Fraction 3 (Methanol): PnT + 500mg/kg of plant extracts

Seven days suppressive test against chloroquine sensitive *P. berghei* NK 65 infection in mice was employed. The mice were randomly divided into five groups. Three groups with 7 mice each were the control groups, while two groups of 8 mice each were the test groups (the ones that the extract was administered). On the first day of the experiment (termed "day 0"), all mice were injected intraperitoneally with *P. berghei* NK 65 infected erythrocytes except the mice in the uninfected/normal control group

Two hours after the infection of mice, the uninfected control (group 1) and the negative control (parasitized non-treated group 2) was given 0.5ml Carboxyl methyl cellulose (CMC-0.7%). The positive controls (the rest of the four groups) received CMC, 10mg/kg chloroquine, and two tests group were given 250mg/kg and 500mg/kg of Methanol extracts for group 4 and 5 respectively. All administration was carried out orally by means of a gavage for seven consecutive days.

On day 5 of the experiment, blood was collected from the tail of the mice and smeared onto a microscopic slide to make a thin film. The blood films were allowed to dry, fixed with methanol (100%) and stained with Giemsa (10%) for 10 minutes. The stained films were then washed with running water and allowed to dry.

The slides were then examined microscopically using x100 magnification in oil immersion (Chike *et al*., 2007). The parasitemia was determined by containing minimum of eight fields per slide with minimum of 100 Red Blood Cells (RBC) per field. The percentage parasitemia was calculated using the modified Peter and Robinson (1992) formula.

$$\% \text{ parasitemia} = \frac{\text{Number of parasitized RBC} \times 100}{\text{Total number of RBC}}$$

The percentage parasitemia was expressed as mean +- SEM. The average suppression of parasitemia was calculated by comparing the average percentage parasitemia in each group with that of negative control, using the formula of Abosi and Raseroke (2003).

$$A = \frac{(B - C)}{B} \times 100$$

Where, A = Average percentage suppression of parasitemia
B = Average percentage parasitaemia inn the negative control group
C = Average percentage parasitaemia in the test group

2.6 COLLECTION OF SAMPLES

Animals were fasted overnight and sacrificed by cervical dissection on day 7 of the experiment, samples were then collected.

2.6.1 COLLECTION OF BLOOD AND SERUM SAMPLES

Blood was collected from the abdominal aorta and the heart via a syringe into plain sterile bottles (for serum), EDTA tubes (for haematological analysis). Blood samples inside the

plain bottles were allowed to stand for 30 minutes to clot and centrifuge at 1000g for 10minutes at room temperature to obtain serum.

2.6.2 PREPARATION OF TISSUE HOMOGENATES

Liver, spleen, and brain tissues were harvested after sacrificing the animals and placed in plain containers and stored in ice. The rest of the tissues (1g) were homogenized with contained 9ml of normal saline and stored in ice (4^{o}C) to obtain 10% homogenate. The resulting homogenates were centrifuged at 1000g of 10minutes, and the supernatant obtained used for subsequent analysis.

2.7 ASSESSMENT OF LIVER FUNCTION

2.7.1 ASPERTATE AMINOTRANSFERASE (AST) ASSAY (Reitman and Frankel, 1957)

2.7.1.1 PRINCIPLE

α-ketoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ L-glutamate + oxaloacetate

Aspartate aminotransferase (AST) is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine. AST catalyses the transfer of an amino group from L-aspartate to α-ketoglutarate, the product of this reversible transamination reaction being oxaloacetate and L-glutamate.

2.7.1.2 PROCEDURE

- The test tubes were labelled as “reagent blank” and “sample❾
- Using a micropipette, 0.1ml of the sample and 0.5ml of solution RI was added to the test tube labelled “sample”. While 0.1ml of distilled water and 0.5ml of solution RI was added to the test tube labelled “reagent blank”. This was followed by proper mixing and incubation of the mixture for 30 minutes at 37^{o}C

- 0.5ml of solution R2 was added to the test tubes (reagent blank and samples), mixed and allowed to stand for 20 minutes at 25^{o}C

- 5.0ml of sodium hydroxide was then added, mixed and the absorbance of sample was read against the reagent blank at 546nm after 5 minutes

2.7.2 ALANINE AMINOTRANSFERASE (ALT) ASSAY (Reitman and Frankel, 1957)

2.7.2.1 PRINCIPLE

α-ketoglutarate + L-alanine $\xrightarrow{\text{ALT}}$ L-glutamate + pyruvate

Alanine aminotransferase (ALT) is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhyrazine. ALT is cleared by sinusoidal cells in the liver. ALT catalyses the transfer of an amino group from L-alanine to α-ketoglutarate, the product of this reversible transamination reaction being pyruvate and L-glutamate

2.7.2.2 PROCEDURE:

- The test tubes were labelled as "reagent blank" and "sample"
- . Using a micropipette, 0.1ml of the sample and 0.5ml of solution RI was added to the test tubes labelled "sample". While 0.1ml of distilled water and 0.5ml of solution RI was added to the test tube "reagent blank". This was followed by proper mixing and incubation of the mixture for 30 minutes at 37^{o}C.
- 0.5ml of solution R2 was added to the test tube (reagent blank and samples), mixed and allowed to stand for 20 minutes at 25^{o}C
- 5.0ml of sodium hydroxide was then added, mixed and the absorbance of sample was read against the reagent blank at 546nm after 5 minutes
- At timed interval, 0.05ml (50µl) of each standard, control, and sample was added to its respective test tube and mixed gently (deionized water was used as sample for reagent blank), then incubated for 10 minutes at 37^{0}C.

- Following the same sequence as the previous step, 2.5ml of alkaline phosphatase colour developer was added at timed intervals and mixed properly.
- The wavelength of the spectrophotometer was set at 590nm. The spectrophotometer was zeroed with reagent blank and the absorbance of the sample was real.

2.7.3 DETERMINATION OF LACTATE DEHYDROGENASE (LDH) ACTIVITY

2.7.3.1 PRINCIPLE:

This method was developed by Tietz, (1976) and was used in evaluation of Lactate dehydrogenase (LDH) activity in serum.

Pyruvate + NADH + H^+ $\xleftarrow{\text{LDH}}$ L-lacatate + NAD^+

2.7.3.2 PROCEDURE:

The test solution was prepared by adding 0.02ml of the sample (using a pipette) into a clean dry test tube and 1ml of the reagent comprising of R1a and R1b, was measured and added into the test tube. The mixture was incubated at 370c and after 30seconds, the absorbance was read at 340nm. The absorbance values at 1,2 and 3 minutes were taken and recorded

2.7.4 TOTAL BILIRUBIN

2.7.4.1 PRINCIPLE:

Calorimetric method based on that described by Jendrassik and Grof (1938). Total bilirubin is determined in the presence of caffeine which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

2.7.4.2 PROCEDURE:

- The test tubes were labelled as “reagent blank” and “sample”.

- Using a micropipette, 0.2ml of the sample and 0.2ml of solution RI was added to the test tubes labelled “blank” and “sample” respectively.
- This is them followed by the addition of 0.2ml of sample solution to both test tubes. Mix and incubate the mixtures for 10mins at 20^0C to 25^0C.
- 1ml of solution of R4 was then added to the test tubes labelled “blank” and “sample” respectively. Mix and incubate for further 5-30mins at 25^0C.

- The wavelength of the spectrophotometer was set at 630nm. The absorbance of the sample and standard were measured against the reagent blank.

2.7.4.3 CALCULATION:

Total Bilirubin concentration (g/L) = $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$ = Standard concentration (g/L)

2.7.5 DETERMINATION OF ALBUMIN

This was determined by the method of Doumas *et al.* (1971).

2.7.5.1 PRINCIPLE:

The estimation of albumin concentration in the serum is on the basis of its quantitative binding to 3, 3′, 5, 5′- tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG) (indicator). At 578 nm, the albumin-BCG-complex is known to absorb at a maximum capacity and the concentration of albumin is directly proportional to the absorbance read.

2.7.5.2 PROCEDURE:

Exactly 0.01 mL of distilled water was mixed with 3.00 mL of BCG reagent (blank). The standard solution (0.01 ml) was also mixed with 3 ml of BCG reagent (standard). Serum (0.01 ml) was mixed with 3.00 mL of BCG reagent (R1). The separate mixtures were incubated for 5 minutes at 20 - 25°C. The absorbance of the sample (A_{sample}) and of the standard ($A_{standard}$) was estimated against the reagent blank at 630 nm.

2.7.6 ESTIMATION OF MALONDIALDEHYDE

This was determined using the protocol explained by Buege and Aust (1987).

2.7.6.1 PRINCIPLE:

Malondialdehyde (MDA) which is a catabolic product of polyunsaturated fatty acids functions as a suitable index in the estimation of the degree of peroxidation reaction. A

product with pink coloration which absorbs maximally at 535 nm is formed when MDA is heated under acidic atmosphere with 2-thiobarbituric acid (TBA).

2.7.6.2 PROCEDURE:

Aliquots of appropriate homogenate supernatants (0.5 ml) were pipetted into the sample test tubes while 0.5 ml of distilled water was pipetted into the blank test tube. Subsequently, 3ml of (1:1:1 v/v/v) TCA-TBA-HCl reagent (TBA 0.375 % w/v, 15 % TCA w/v and 0.25 N HCl) was added across all test tube and mixed thoroughly by swirling. The solution was heated for 15 minutes in a boiling water bath at 100 °C. After cooling, the flocculent precipitate was removed by centrifugation at 1000 g for 10 minutes. The absorbance of the clear supernatant was measured against a reference blank at 535nm. The MDA concentration of the sample was calculated using an estimation coefficient of 1.56×10^5 m^{-1} cm^{-1} according to the expression of Adam-Vizi and Seregi (1982).

CHAPTER 3

3.0 RESULTS

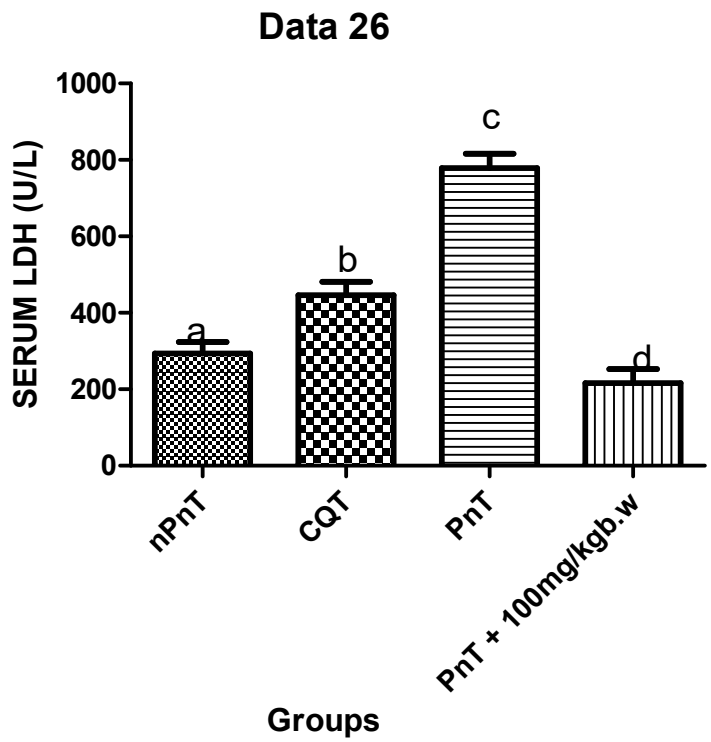


FIGURE 1: Serum lactose dehydrogenase level

nPnT which represents the uninfected mice shows the normal of LDH in the mice, infection of the mice with the parasite caused a significant increase in the LDH level. Chloroquine reduced the amount of LDH significantly. Then 100mg/kg b.w dosage of the plant extract reduced the LDH level in the serum of infected mice.

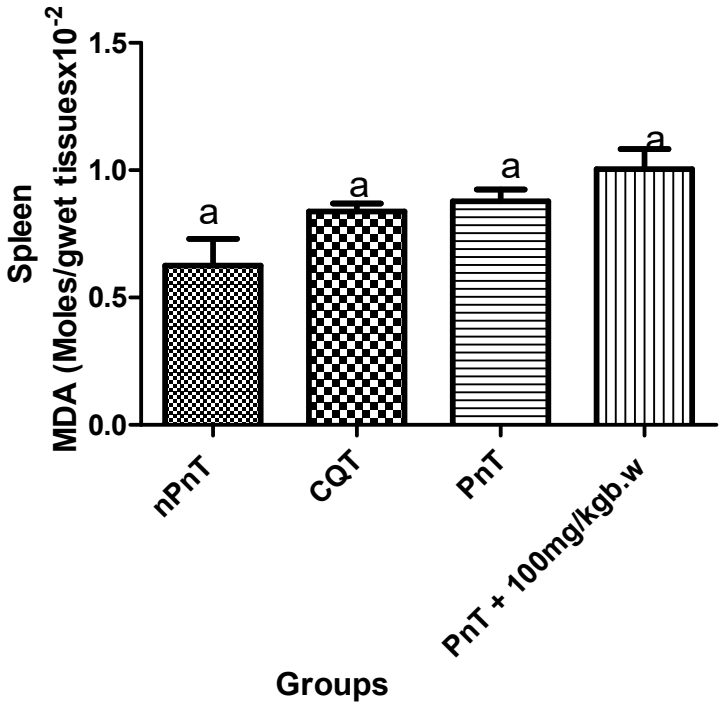


FIGURE 2: Maloniadehyde test result

MALONDIALDEHYDE (MDA)

The normal control group (nPnT) showed the normal level of MDA in the spleen. Infection of the parasite did not cause a significant change in the level of MDA. Chloroquine treated mice and the mice treated with 100m/kg b.w showed the same level of MDA as the non-parasitized mice.

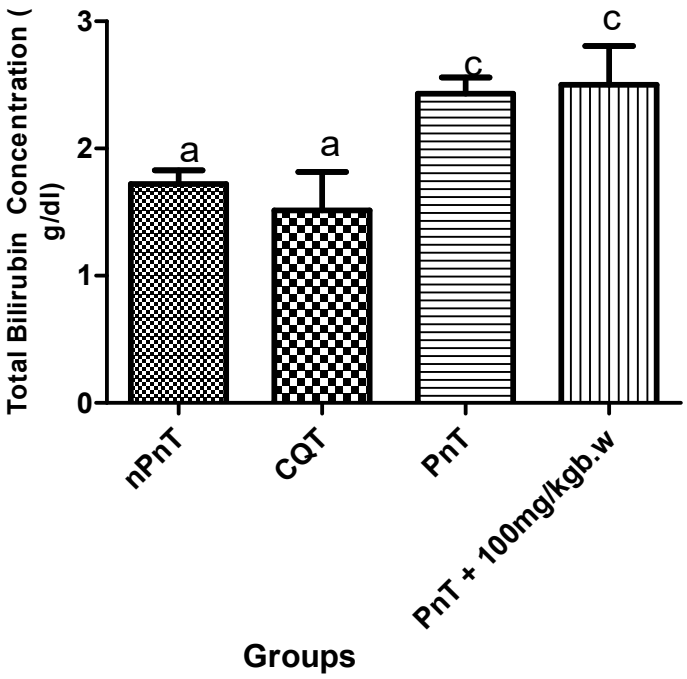


FIGURE 3: Aspartate transaminase concentration

nPnT shows the normal level of Total Bilirubin, the infection of the plasmodium parasite causes a significant increase in the Total Bilirubin level. Chloroquine treatments reduce the level of bilirubin in the infected mice to the same level of the non-infected mice (nPnT). 100mg/kg b.w of the plant extract causes no reduction in the level of bilirubin in infected mice.

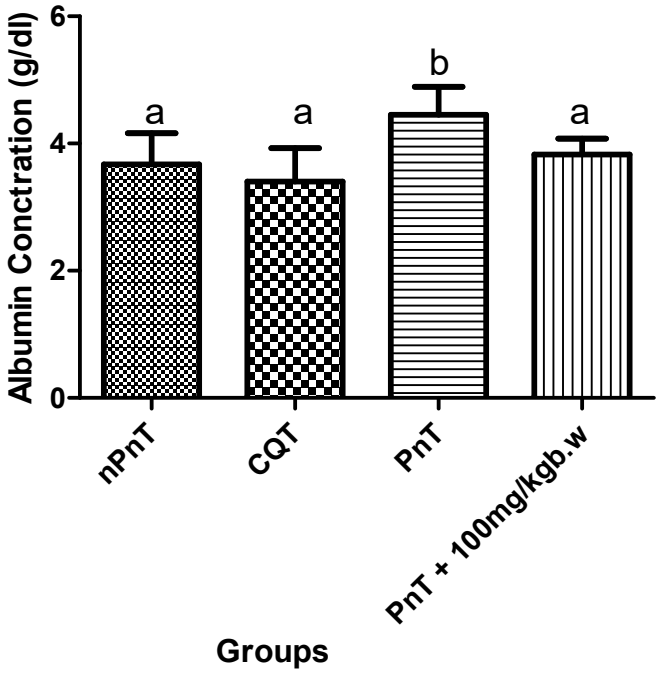


FIGURE 4: Albumin concentration

nPnT shows the normal levels of Albumin in non-infected mice. The Albumin level increases slightly in the PnT. CQT and 100mg/kg b.w dosage of plant extract shows the same level of Albumin. However, treatment with 100mg/kg b.w of extract significantly increased the levels of Albumin concentration when compared with the positive control.

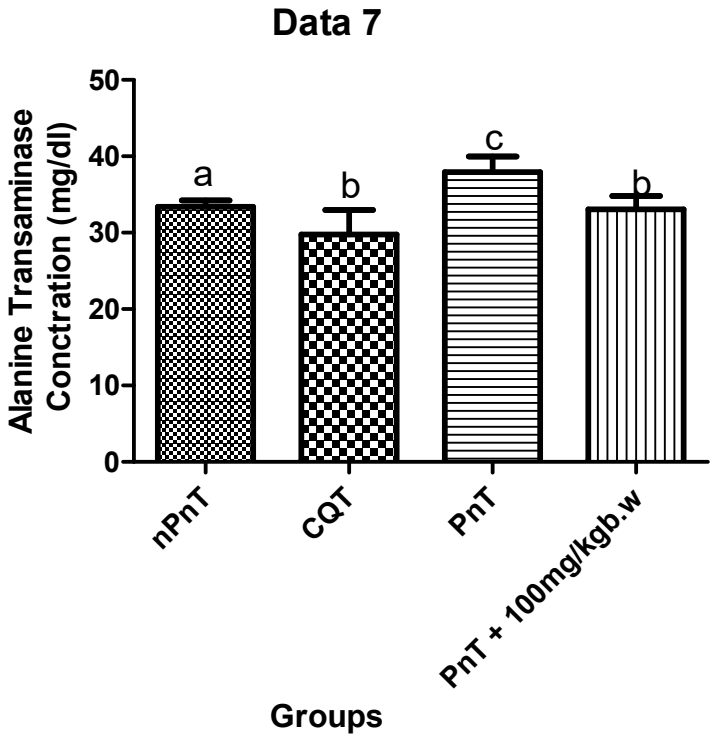


FIGURE 5: Total bilirubin concentration

nPnT shows the normal level of Alanine Transaminase (ALT) in the serum. Infected mice show an increase in the ALT level in the serum. CQT reduces the ALT to the normal range of the nPnT mice, 100mg/kg b.w of the plant extract shows a slight decrease in ALT levels.

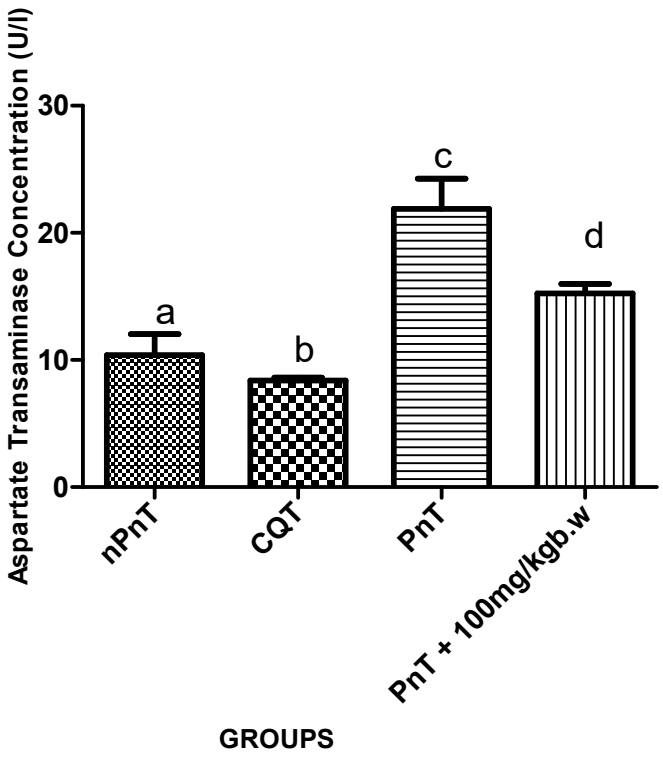


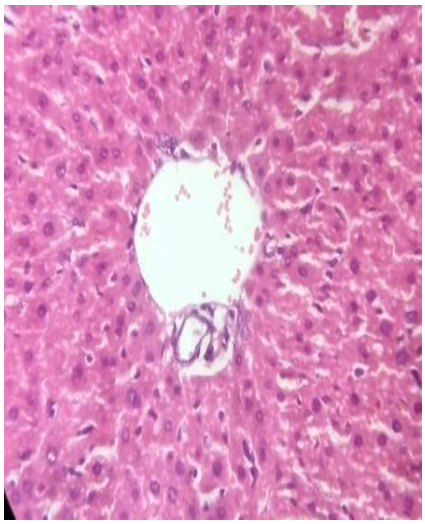
FIGURE 6: Alanine transaminase concentration

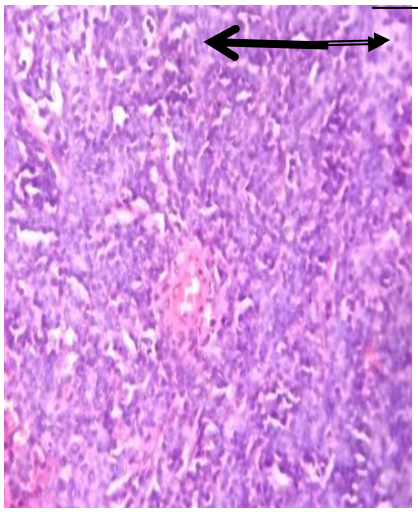
nPnT shows the normal level of Aspartate Transaminase (AST) in the serum, the parasite infected mice show an increased level of serum AST. CQT treatment reduces the AST level in the serum significantly, 100mg/kg b.w dosage of the plant extract reduces the AST serum level

Histopathology

nPnT group

LIVER SPLEEN

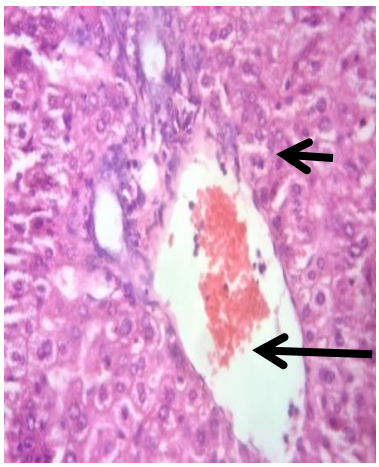


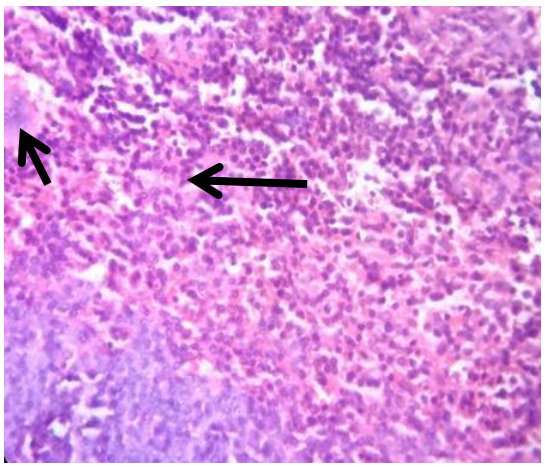


Liver histology of the healthy mice group reveals visible centriole (long arrow) and well fenestrated sinusoids and hepatocytes (short arrow). The Spleen histology reveal white and red pulp with prominent central artery (long arrow) and prominent lymphoctes with that appears granulated (short arrow).

PnT group

LIVER SPLEEN

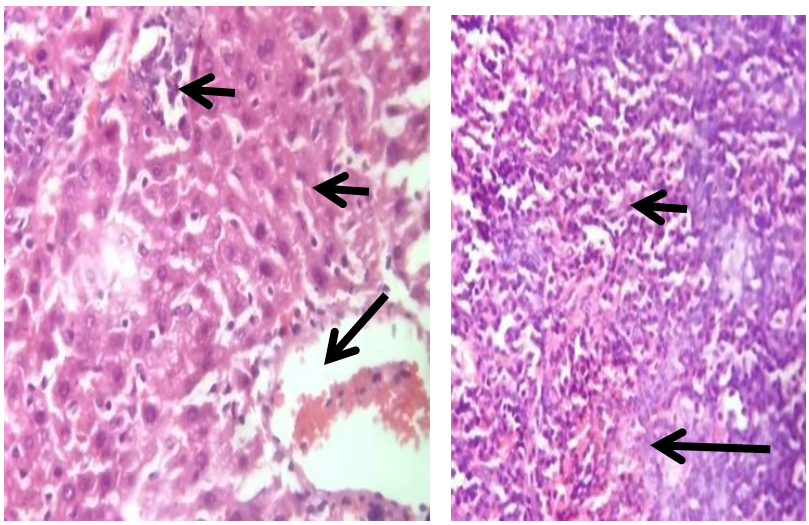




Liver histology of the infected non treated mice reveals visible centriole with mildly thickened wall surrounded by focal mononuclear exudates (long arrow) and well fenestrated sinusoids and hepatocytes (short arrow). The Spleen histology reveal white and red pulp with not so prominent central artery (long arrow) and prominent lymphoctes with with apoptic changes and tangible macrophages that appears granulated (short arrow).

CQT group (chloroquine treated group)

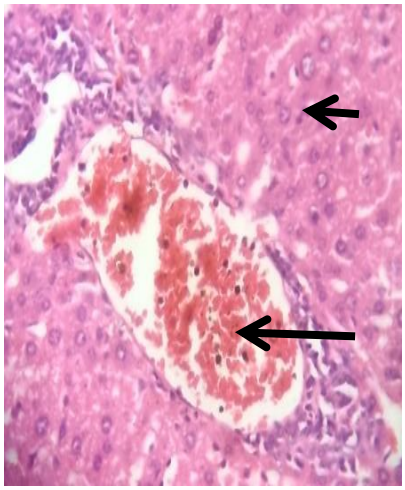
LIVER SPLEEN

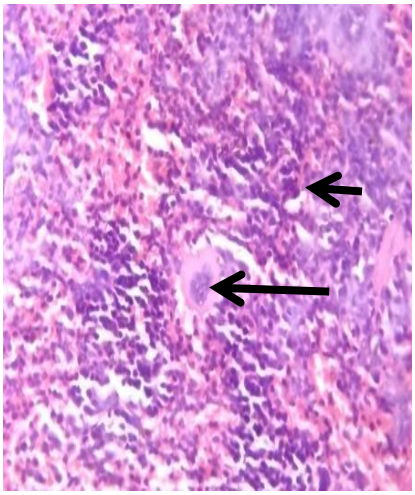


Liver histology of the chloroquine treated mice reveals visible mildly congested centriole surrounded by mononuclear exudates (long arrow) and well fenestrated sinusoids and hepatocytes with dispersed and focal mononuclear inflammatory cells (short arrow). Spleen histology reveal white and red pulp with no prominent central artery (long arrow) and lymphoctes with with apoptic changes and tangible macrophages that appears granulated (short arrow).

PnT × 100mg/kg b.w group

LIVER SPLEEN





Liver histology of the 100mg/kg b.w of plant of plant extract treated mice reveals visible congested dilated centriole surrounded by thickened exudates of mononuclear and inflammatory exudates (long arrow) and well fenestrated sinusoids and hepatocytes (short arrow). Spleen histology reveal white and red pulp with visible central artery (long arrow) and lymphoctes with with apoptic changes and tangible macrophages that appears granulated (short arrow).

CHAPTER 4

4.0 DISCUSSION

An earlier study's phytochemical investigation of *D. microcarpum* found that it included triterpenes, alkaloids, glycosides, flavonoids, and cardiac glycosides in addition to saponins, sugars, and tannins. (Abreu *et al*., 1998 ; Abubakar *et al*., 2017 ; David J *et al*. 2017)

Alkaloids, saponins, tannins, flavonoids, glycosides, and triterpenes, which have a wide range of therapeutic applications, were discovered in this study's methanol stem bark extract of *D. microcarpum*. To set the dose to be utilized in the future investigation, an acute toxicity study is carried out to identify the dose that results in mortality or significant toxicity in 50% of the animals within a given time. (Yaro AH *et al.,* 2017)

When assessing the safety or toxicity of a new chemical, it is crucial to be aware of the state of the kidney and liver. The liver is a crucial organ for drug metabolism and disposal, and the activities of several serum indicators, such as ALT, AST, ALP, total protein, albumin, and bilirubin, can be used to assess the liver's proper function. As is well known, AST is a cytoplasmic and mitochondrial enzyme present in a variety of tissues, while ALT is a liver enzyme (liver, kidney, heart, skeletal muscles, and brain). (Onu A *et al.,* 2013). Hepatocellular injury is indicated by elevated ALT and AST values. w

The liver is the location of medication metabolism and elimination. The liver function test gauges the liver's efficiency in carrying out its fundamental duties of producing protein and removing bilirubin. The liver's regular functions are disrupted by the plasmodium parasite, which first infects the liver cells. The PnT group tested the effects of the plasmodium parasite on the liver of mice while experimenting with the effects of 100 mg/kg b.w. of the sweet dattock plant on suppressing the plasmodium parasite.

The blood serum and liver were subjected to AST, ALT, Albumin, Total Bilirubin, and antioxidant assays to confirm the sweet dattock plant's antiplasmodial effects. The levels of AST and ALT in the serum were dramatically raised by plasmodium parasite infection, however 100mg/kb b.w of the plant extract could lower these levels, suggesting that it had an impact on the liver's functionality. (Peter IT, Anatoli VK 1998)

The blood stage of the plasmodium parasite caused greater hemolysis of the red blood cells, which is why the level of bilirubin increased dramatically in the PnT group. However, there

was no change in the infected mice's bilirubin levels after receiving 100 mg/kg b.w., suggesting that the plant extract was unable to stop the hemolysis of red blood cells.

An enzyme called LDH is released when tissue is damaged. The blood serum of the infected mice (PnT) showed a rise in LDH levels, a sign of liver injury. LDH levels in the serum were significantly reduced after receiving 100mg/kg b.w. of the plant extract. This suggested that it significantly affected the healing of tissue injury.

To assess oxidative stress, MDA was utilized. The imbalance between the body's levels of reactive oxygen species and antioxidants, which causes damaged cells and tissues, is the cause of this oxidative stress. The lipid peroxidation process in an organism is initiated by free radicals. One of the byproducts of polyunsaturated fatty acid peroxidation in cells is malondialdehyde (MDA). A rise in free radicals leads to an excess of MDA generation. The fact that MDA levels increased slightly after taking the plant extract at a dose of 100 mg/kg b.w. indicates that the medicine had no impact on the decrease of oxidative stress.

Toxic chemicals can easily target the hematopoietic system (Adeneye AA *et al.,* 2006). To assess the negative effects of foreign substances, such as plant extracts, on blood contents, hematological indicators can be used (Ashafa AOT *et al*., 2012). The studied hematological parameters showed no significant difference between the treatment groups and the control group. The observed differences may indicate that the extract is safe at the measured levels even though they have little toxicological significance.

4.1 CONCLUSION

The ethnomedicinal use of *D. microcarpum* in the treartment of malaria infection is supported by the fact that the plant’s stem bark extract in methanol exhibits significant antiplasmodial activity. After brief exposure, the evaluation of biochemical and hematological indicators indicates that the extract generally safe at the levels examined.

REFERENCES

Abdalbasit, A. M., Mohamed E.S., Mirghani, A.B.A. and Siddig, I.A. (2009). "Detarium microcarpum Guill and Perr fruit proximate chemical analysis and sensory characteristics of concentrated juice and jam," African Journal of Biotechnology. 8(17): 4217-4221.

Abreu EL, Cheng A, Kelly PJ, Chertoff K, Brotto L, Griffith E, et al. Skeletal muscle troponin as a novel biomarker to enhance assessment of the impact of strength training on fall prevention in the older adults. Nurs Res. 2014

Abreu, P.M., Rosa, V.S., Araujo, E.M., Canda, A.B., Kayser, O., Bindseil, K.U., Siems, K. and Seeman, A. (1998). Phytochemical analysis and antimicrobial evaluation of Detarium microcarpum bark extracts. Pharmaceutical and Pharmacological letters. 8(3):107-109.

Africa Union Memoir, author. "Unite against Malaria, together we can beat malaria" on African Malaria Control Day. World Malarial Report (2005) 2005 Information available to WHO and UNICEF at the end of 2004.

Airaodion, I., Ibrahim, A.H., Ogbuagu, U., Ogbuagu, E.O. and Awosanya, O.O. (2009). Evaluation of phytochemical content and antioxidant potential of Ocimum gratissimum and Telfairia occidentalis leaves. Asian J Res Med Pharmaceut Sci. 7: 1-11.

Akinmoladun, A.C., Ibukun, E.O., Afor, E., Obuotor, E.M. and Farombi, E.O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. Sci Res Essay. 2 : 163-166.

Al-Massarani, S.M., El-Gamal, A.A., Parvez, M.K. et al. (2017). New cytotoxic seco-type triterpene and labdane-type diterpenes from Nuxia oppositifolia. Molecules. 22(3):389.

Annan, Z. et al. (2007). Population genetic structure of Plasmodium falciparum in the two main African vectors. Anopheles gambiae and Anopheles funestus. Proc. Natl Acad. Sci. USA 104, 7987–7992.

Baker, D.A. (2010). Malaria gametocytogenesis. Mol. Biochem. Parasitol. 172: 57–65.

Bankole, A.E., Adekunle, A.A., Sowemimo, A.A., Umebese, C.E., Abiodun, O. and Gbotosho, G.O. (2016). Phytochemical screening and in vivo antimalarial activity of extracts from three medicinal plants used in malaria treatment in Nigeria. Parasitol Res. 115:299–305.

Bankole, A.E., Adekunle, A.A., Sowemimo, A.A., Umebese, C.E., Abiodun, O. and Gbotosho, G.O. (2016). Phytochemical screening and in vivo antimalarial activity of

extracts from three medicinal plants used in malaria treatment in Nigeria. Parasitol Res. 115:299–305.

Bantie, L., Assefa, S., Teklehaimanot, T. and Engidawork, E. (2014). In vivo antimalarial activity of the crude leaf extract and solvent fractions of Croton macrostachyus Hocsht. (Euphorbiaceae) against P. berghei in Mice. BMC Complement Altern Med. 14:79.

Bhatt, S. et al. (2015). The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature. 526: 207–211.

Boddey, J. A. and Cowman, A. F. (2013). Plasmodium nesting: remaking the erythrocyte from the inside out. Annu. Rev. Microbiol. 67: 243–269.

Bouyate, A.M. and Sam D.P. (2006). Detarium microcarpum Guill & Perr. In: Schmetrer GH, Gurib Fakim A Edn. Prota (11)1: Medicinal plants/plantesmeduinales I (CD-Rom) PROTA, Wageningen, Netherlands.

Cavin, A.L., Hay, A.E., Marston, A., Stoeckli-Evans, H., Scopelliti, R., Diailo, D. and Hostelfman, K. (2006). Bioactive diterpenes from the fruits of Detarium microcarpum. Journal of NaturalProducts. 69(5): 768-773.

Cheng, Q. et al. Plasmodium falciparum parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting. Malar. J. 13, 283 (2014).

Cheng, Y. et al. (2016). Plasmodium vivax GPI-anchored micronemal antigen (PvGAMA) binds human erythrocytes independent of Duffy antigen status. Sci. Rep. 6: 355-381.

Chikezie, P.C., Ojiako, O.A. and Nwufo, K.C. (2015). Overview of anti-diabetic medicinal plants: The Nigerian research experience. J Diabetes Metab. 6: 546-547.

Cui, L., Mharakurwa, S., Ndiaye, D., Rathod, P.K. and Rosenthal, P.J. (2015). Antimalarial drug resistance: literature review and activities and findings of the ICEMR network. Am J Trop Med Hyg. 93 (3):57–68.

Daily, J. P. (2017). Malaria 2017: update on the clinical literature and management. Curr. Infect. Dis. Rep. http://dx.doi.org/10.1007/s11908-017-0583-8.http://apps.who.int/iris/bitstream/10665/162441/ 1/9789241549127_eng.pdf (2015).

Dalziel, J.M. (1937). The useful plants of west tropical Africa. Gown Agents for Overseas Colonies London.

Dugacki, V. (2005). Dr. Rudolf Battara operation in Nin in 1902, the first systematic battle attempt against malaria in Croatia. Med. Jaderina. 35: 33-40.

Ebi, G.C. and Afieroho, O.E. (2011). Phytochemical and antimicrobial studies on Detarium microrcarpum Guill and Perr (Caesalpiniaceae) seeds coats. African Journal of Biotechnology. 11(3): 457- 462.

Ebohon, O., Irabor, F., Ebohon, L.O. and Omoregie, E.S. (2019). Therapeutic failure after regimen with artemetherlumefantrine combination therapy: a report of three cases in Benin City, Nigeria. Rev Soc Bras Med Trop. 52:20190163.

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005). Phytochemical Constituents of some Nigerian Medicinal Plants. Afr. J. Biotechnol. 4(7): 685-688.

Elguero, E. et al. (2015). Malaria continues to select for sickle cell trait in Central Africa. Proc. Natl Acad. Sci. USA. 112: 7051–7054.

Falodun, A., Chaudhry, A. M. A. and Choudhary, I. M. (2009). Phytotoxic and Chemical Investigations of a Nigerian Medicinal Plant. Res. J. Phytochem. 3: 13-17.

Federal Ministry of Health. (2009d). National framework for monitoring and evaluation of malaria control in Nigeria, Federal Ministry of Health. Abuja, Nigeria. pp. 39.

Florens, L. et al. (2002). A proteomic view of the Plasmodium falciparum life cycle. Nature. 419: 520-526.

Frischknecht, F. et al. (2004). Imaging movement of malaria parasites during transmission by Anopheles mosquitoes. Cell. Microbiol. 6: 687–694.

Gamboa, D. et al. (2010). A large proportion of P. falciparum isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. PLoS ONE, 5: 8091.

Gunawan CA, Harijanto PN, Nugroho A. Quinine-induced arrhythmia in a patient with severe malaria. Acta Med Indonesia. 2007

Joanny, F., Lohr, S. J., Engleitner, T., Lell, B. and Mordmuller, B. (2014). Limit of blank and limit of detection of Plasmodium falciparum thick blood smear microscopy in a routine setting in Central Africa. Malar. J. 13: 234.

Karunaweera, N. D., Grau, G. E., Gamage, P., Carter, R. and Mendis, K. N. (1992). Dynamics of fever and serum levels of tumor necrosis factor are closely associated during clinical paroxysms in Plasmodium vivax malaria. Proc. Natl Acad. Sci. USA. 89: 3200–3203.

Kouyate, A.M. (2005). Aspects ethnobotaniqueset etudea de la variability morphologique biochemique et phenologuque de Detarium microcarpum, Guill & PCN. AU. Mali. Ph.D Thesis, Faculty of Agriculture and Applied Biological Sciences, University of Kent Belgium.

Kwiatkowski, D. P. (2005). How malaria has affected the human genome and what human genetics can teach us about malaria. Am. J. Hum. Genet. 77:171–192.

Lambert RK, Wiggs BR, Kuwano K, Hogg JC, Paré PD. Functional significance of increased airway smooth muscle in asthma and COPD. J Appl Physiol. 1993

Lcung, W.T., Busson, F. and Jardin, C. (1968). Food consumption table for; use in Africa, FAO, Rome Italy p. 306.

Lehrer, S. (1981). Vitezi Medicine; Prosveta: Beograd, Serbia.

Lim, C. et al. (2016). Reticulocyte preference and stage development of Plasmodium vivax isolates. J. Infect. Dis. 214:1081–1084.

Llanos-Cuentas, A. et al. (2014). Tafenoquine plus chloroquine for the treatment and relapse prevention of Plasmodium vivax malaria (DETECTIVE): a multicentre, double-blind, randomised, phase 2b dose-selection study. Lancet. 383:1049-1058.

Lopez, C., Saravia, C., Gomez, A., Hoebeke, J. and Patarroyo, M. A. (2010). Mechanisms of genetically-based resistance to malaria. Gene. 467: 1-12.

Mahmood, N., Pizza, C., Aquino, P., de Tommasi, N., Piacente, S., Colman, S., Buma, A. and Itay, A.J. (1993). Inhibition of HIV infection by flavanoids. Antiviral Research. 22: 189-199. pathogenesis: insights for new treatments. Nat. Med. 19: 156-167.

Miller, L. H., Ackerman, H. C., Su, X. Z. and Wellems, T. E. (2013). Malaria biology and disease pathogenesis: insights for new treatments. Nat. Med. 19: 156-167.

Mishra SK, Mohanty S, Satpathy SK, Mohapatra DN. Cerebral malaria in adults: a description of 526 cases admitted to Ispat General Hospital in Rourkela. India. Ann Trop Med Parasitol. 2007

Mitchell, S. N. et al. (2015). Mosquito biology. Evolution of sexual traits influencing vectorial capacity in anopheline mosquitoes. Science. 347: 985-988.

Murithi, C.K., Fidahusein, D.S., Nguta, J.M. and Lukhoba, C.W. (2014). Antimalarial activity and in vivo toxicity of selected medicinal plants naturalised in Kenya. Int Educ Res J. 2:395-406.

Nkogbe, D., Akue, J.P., Gonzalezand, J.P. and Leroy, E.M. (2011). Prevalence of Plasmodium falciparum infection in submicroscopic infection. Pakistan; District Zhob. Pakistan Journal of Biological Science. 11(12): 1620-1624.

Nogueira HM, Gama RD. Bull′s-eye maculopathy. N Engl J Med. 2009

Nwaogo, L.A., Aliasi, C.S., Ibegulum, C.O. and Igwe, C.U. (2007). Afr J Biotech. 6(7): 890-893.

Okell, L.C.T., Bousema, T., Griffin, J.T., Ouedraogo, A.L., Ghani, A.C. and Drakeley, C.J. (2012). Factors determining the occurrence of submicroscopic malaria infections and their relevance foe control. Nature communications. 3:1237.

Okolo, C.E., Akah, P.A. and Uzodinma, S.U. (2012). Antidiabetic activity of root extract of Detarium microcorpum (Fabiacase) Quill & Perr. Phytopharmacology. 3 (1): 12-18.

Olusegun N.A. and Onye, K.N. (2013). Possible ACT resistant malaria in Nigeria – A report of 3 cases. Rev Soc Bras Med Trop. 46(4):525–527.

Ouédraogo, A. L. et al. (2015). Efficacy and safety of the mosquitocidal drug ivermectin to prevent malaria transmission after treatment: a double-blind, randomized, clinical trial. Clin. Infect. Dis. 60: 357–365.

Parroche, P. et al. (2007). Malaria hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to Toll-like receptor 9. Proc. Natl Acad. Sci. USA. 104: 1919–1924.

Paul, A. S., Egan, E. S. and Duraisingh, M. T. (2015). Host–parasite interactions that guide red blood cell invasion by malaria parasites. Curr. Opin. Hematol. 22: 220–226.

Paul, A. S., Egan, E. S. and Duraisingh, M. T. (2015). Host–parasite interactions that guide red blood cell invasion by malaria parasites. Curr. Opin. Hematol. 22: 220–226.

Phillips, M. A. (2011). in Neglected Diseases and Drug Discovery (eds Palmer, M. and Wells, T. N. C.) 65–87.

Regillo CD. Basic and clinical science course, section 12: retina and vitreous 2011

Rosenberg, R., Wirtz, R. A., Schneider, I. and Burge, R. (1990). An estimation of the number of malaria sporozoites ejected by a feeding mosquito. Trans. R. Soc. Trop. Med. Hyg. 84: 209–212.

Rugemalila, J.B., Wanga, C.L. and Kilama, W.L. (2006). Sixth Africa Malaria day in 2006: how far have we come after the Abuja declaration? Malaria Journal. 5: 102.

Sinnis, P. and Zavala, F. (2012). The skin: where malaria infection and the host immune response begin. Semin. Immunopathol. 34:787–792.

Smith AL. Text on Microbiology and Pathology. Eleventh edition. USA: Published by CV Mosby Com.; 1978

Smith, J. D. (2014). The role of PfEMP1 adhesion domain classification in Plasmodium falciparum pathogenesis research. Mol. Biochem. Parasitol. 195: 82–87.

Spillman, N. J. and Kirk, K. (2015). The malaria parasite cation ATPase PfATP4 and its role in the mechanism of action of a new arsenal of antimalarial drugs. Int. J. Parasitol. Drugs Drug Resist. 5: 149-162.

Tan SS, Ong B, Cheng C, Ho WE, Tam JK, Stewart AG, Harris T, Wong WS, Tran T. The antimalarial drug artesunate inhibits primary human cultured airway smooth muscle cell proliferation. Am J Respir Cell Mol Biol. 2014

Tang, H., Tan, Z. and Xu, W. (2014). Immune evasion strategies of pre-erythrocytic malaria parasites. Mediators Inflamm. 2: 36-40

Tönnesmann E, Kandolf R, Lewalter T. Chloroquine cardiomyopathy a review of the literature. Immunopharmacol Immunotoxicol. 2013

Verlinden BK, Louw A, Birkholtz LM. Resisting resistance: is there a solution for malaria? Expert Opin Drug Discov. 2016

White, N.J.N., Pukrittayakamee, S., Hien, T.T.T., Faiz, M.A., Mokuolu, O.A.O. and Dondorp, A.A.M. (2014). Malaria. Lancet. 383: 723-735.

World Health Organization. Guidelines for the treatment of malaria, 3rd edn. WHO http://apps.who.int/iris/bitstream/10665/162441/ 1/9789241549127_eng.pdf (2015).

World Health Organization. Guidelines for the treatment of malaria, 3rd edn. WHO

World Health Organization. Malaria: diagnostic testing. WHO http://www.who.int/malaria/areas/ diagnosis/en/ (2016).

World Malarial Report. 2005 Information available to WHO and UNICEF at the end of 2004.

Wundermann, G.S. and Osiki, A.A. (2017). Currently observed trend in the resistance of malaria to artemisinin-based combination therapy in Nigeria – a report of 5 cases. Int j trop dis health. 21(2):1-5.