ANTIMALARIAL AND ANTIOXIDANT EFFECTS OF METHANOL AND FLAVONOID-RICH EXTRACTS OF *Adansonia digitata* STEM BARK ON *Plasmodium berghei*-INFECTED MICE

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ABSTRACT

Adansonia digitata has popular ethnomedicinal application in the treatment of malaria in sub-Saharan Africa. The present study sought to investigate the antimalarial and antioxidant effects of methanol and flavonoid-rich extracts of the stem bark on *Plasmodium berghei*-infected mice *in vivo*. Thirty-five male mice, weighing 18-20 g and randomly allocated into seven groups of five animals each were used. Group 1, which served as the positive control, was pretreated with 1 ml/kg of the vehicle (5% v/v tween 80), mice in groups 2 and 3 were pretreated with 5 mg/kg b.wt of the standard drugs: chloroquine and arthemter/lumfantrine respectively, groups 4 and 5 were pretreated with 200mg/kg and 400 mg/kg methanol extract of *A. digitata* (ADME) respectively while groups 6 and 7 were administered 200mg/kg and 400 mg/kg flavonoid-rich extract of *A. digitata* (ADFE)respectively. Drugs were administered by oral gavage once daily for five consecutive days before intraperitonial transfection of mice with an inoculum size of 1x 10^7 of *P.berghei*. Blood was withdrawn from animals for the quantification of packed cell volume (PCV) and parasitemia. Animals were anaesthetized with ether 72 h after transfection, dissected and the livers quickly excised to prepare the homogenate used to evaluate the extent of membrane lipid peroxidation and level of reduced glutathione (GSH).

ADME and ADFE treatment caused significant (P<0.001), dose-dependent chemosuppresive activity and decreased parasitemia when compared with the infected, untreated mice. Higher effective doses (400 mg/kg b.wt) of ADME and ADFE produced 68% and 82% clearance of the parasites at day 5 after transfection compared with 100% clearance by both standard drugs (chloroquine and arthemter/lumfantrine) at 5 mg/kg b.wt dosage. ADME and ADFE at 400 mg/kg b.wt also reversed the malaria-dependent reduction in the PCV to pre-infection level and compared well with the reference drugs in this regard. ADME and ADFE at

the evaluated concentrations significantly (P<0.001) reversed the elevated hepatic membrane peroxidation caused by *P. berghei* infection but produced no significant effect on GSH when compared with the infected, untreated mice.

The results of the present study revealed the antioxidant and prophylactic effects of methanol and flavonoid-rich extracts of *A. digitata* on *P. berghei*-induced malaria in mice. It can thus be concluded that the plant could be harnessed as source of antimalarial agents and further justifies the folkloric use of the plant in the treatment of malaria.

CERTIFICATION

This is to certify that this research work "Antimalarial and Antioxidant Effects of Methanol and Flavonoid-Rich Extracts of *Adansonia digitata* Stem Bark on *Plasmodium berghei*-Infected Mice" was carried out by **OLATUNDE Moses Damilola (BTH/11/0259)** under my supervision in the Department of Biochemistry, Federal University, Oye-Ekiti and that this work has not been submitted elsewhere for the award of a degree.

Supervisor`s Name: **Dr. K. Komolafe**

Signature……………………….   Date…………………….

Head of Department`s Name: **Dr. R.E., Okonji**

Signature……………………….   Date…………………….

DEDICATION

This work is dedicated to the Almighty God, the source of my life's journey. To my wonderful Parents who had faith in me and gave me a chance. To my siblings who through thick and thin, held on to the quasi faint hope. To Pastor and Mrs. Ajewole, for never hesitating to lend a helping hand.

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My profound gratitude to Pastor and Mrs. Ajewole, for their love, care, prayers, advice, words of encouragement and support, in pushing me towards the set goals. It would have been impossible without you. For your big heart, I am saying a big thank you in return.

My heartfelt appreciation goes to my lovely parents, they birthed the idea, their love, care and prayers would not stop pushing me towards the horizon. Your faith in me kept me encouraged throughout the journey. At odd times, remembering you makes me beam. My siblings were indeed great, the success of this work wouldn't have been easy without you all, bearing it all, as well as sparing that "seemingly little" moves me several paces forward.

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CHAPTER ONE

1.0   INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals. It is a life-threatening blood disease caused by a Plasmodium parasite. The disease is transmitted most commonly by an infected female Anopheles mosquito. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood (WHO, 2014). The parasites travel to the liver where they mature and reproduce. Five species of *Plasmodium* can infect and be spread by humans. Most deaths are caused by *P. falciparum* because *P. vivax*, *P. ovale*, and *P. malariae* generally cause a milder form of malaria. The species *P. knowlesi* rarely causes disease in humans (Caraballo, 2014; WHO, 2014).

Malaria is the most important parasitic disease of man. This disease is presently endemic and it is a major threat to public health in various parts of the world, around the equator and areas such as parts of Asia, Latin America, Middle East, Eastern Europe, Pacific and much of Africa. It is largely prevalent in these places and specifically accounts for 85-90% of fatalities in the Sub Saharan Africa (Layne, 2007). The prevalence of malaria in the tropical and subtropical regions have been attributed to rainfall, consistent high temperatures and high humidity as well as the presence of stagnant waters in which mosquito larvae readily mature, thus providing a favourable environment for the continuous breeding of this vector (Jamieson *et al.*, 2006).

This disease is reasonably easy to recognize especially in patients with little or no previous case(s) of malaria. The common symptoms include headache, fever, shivering, joint pain, vomiting, hemolytic anemia, and jaundice, hemoglobin in the urine, convulsions and retinal damage (Beare *et al.,* 2006).

The signs and symptoms of malaria typically begin 8–25 days following infection (Fairhurst and Wellems, 2010). It was however suggested that symptoms may occur later in those who have taken antimalarial medications as prevention (Nadjm and Behrens, 2012). Malaria can be diagnosed by the microscopic examination of a patient's stained blood film. This disease is often treated with antimalarial drugs depending on its type and severity. Uncomplicated malaria may be treated with oral medications. The most effective treatment for *P. falciparum* infection is the use of artemisinins in combination with other antimalarials (known as artemisinin-combination therapy, or ACT), which decreases resistance to any single drug component (Kokwaro, 2009).

Plant derived foods contain many bioactive compounds in addition to those which are traditionally considered as nutrients, such as vitamins and minerals. These physiologically active compounds, referred to simply as 'phytochemicals', are produced via secondary metabolism in relatively small amounts (Rodriguez *et al.,* 2006). Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). They are non essential nutrients; some are responsible for color and other organoleptic properties and may have biological significance (US FDA, 2014).

The number of these phytochemicals has increased greatly over the last decades and those of significant health benefits have been grouped into classes which include alkaloids, terpenes, glycosides, flavonoids, phenolics, saponins, tannins, steroids etc. Phytochemicals exhibit various pharmacological activities i.e. anti-inflammatory, antioxidant, anti-malaria, anti-cancer, anaesthetics, and anti-viral, anti-fungal and anti-bacterial activities (Abdul Wadood *et al.*, 2013; Rodriguez *et al.*, 2006).

Traditional medicines have been in use for the treatment of malaria for thousands of years and are the source of the two main groups (artemisinin and quinine) of modern antimalarial drugs (Kazembe *et al.*, 2012). Medicinal plants contain some phytochemicals (bioactive components) which exert definite physiological actions and are thus responsible for their medicinal properties in curing diseases and herbal preparations account for 30-50% of total medicine consumption (Abdul Wadood *et al.*, 2013; Kazembe *et al.*, 2012). The problems of increasing pathogen resistance, e.g. *Plasmodium* to established antimalarial drugs (e.g. quinine, chloroquine) coupled with the difficulties of the poor populace to afford and access effective antimalarial drugs, have necessitated investigation of chemical compounds from plants for antimalarial properties with the aim of finding novel drugs (Ibrahim *et al.,* 2012). Recent findings from various studies have boosted the confidence in the once abandoned herbs for the treatment of resistant form of malaria parasites (Avwioro, 2010). This has the capacity to change the perspectives on traditional medicine and its role in the health management, paving the way for better collaboration between modern and traditional systems (Graz *et al.,* 2011). Some of the medicinal plants used for treating malaria include *Artermisia annua* (from which artemisinin was obtained), *Enantia chloranta, Carica papaya, Mangifera indica, Psidium guajava, Adansonia digitata* among others.

Adansonia digitata (baobab), a plant of the family of Bombaceae, is the most widespread of the *Adansonia* species on the African continent, found in the hot, dry savannahs of sub-Saharan Africa. In general, baobab is a good medicinal plant. Baobab pulp is rich in vitamin C, the leaves are rich in good quality proteins – most essential amino acids are present in the leaves and minerals, and the seeds in fat. Moreover, pulp and leaves exhibit antioxidant activity (Chadare *et al*., 2009). A variety of chemicals have been isolated and characterized from *A.*

digitata. They belong to the classes of terpenoids, flavonoids, steroids, vitamins, amino acids, carbohydrates and lipids (Donatien *et al.,* 2011). Baobab bark is mainly used for medicinal properties. The bark is thought to contain a bioactive component for treatment of malaria and other fevers (Sidibe and Williams, 2002). Baobab bark which is often given to infants to promote weight gain (Lockett and Grivetti, 2000) was found to be high in fat, calcium, copper, iron, and zinc (Lockett *et al.,* 2000).

A. digitata has a very high content of dietary antioxidant, including polyphenols, vitamin C and E, carotenoids. This makes it effective in preventing oxidative stress related diseases (Besco *et al.,* 2007), such as inflammation, cardiovascular disease, cancer and aging related disorders (Besco *et al.,* 2007).

Evidences has also confirmed the use of the extracts of the leaves, fruits, seeds and bark as an antimicrobial, antiviral (De Caluwe *et al.*, 2010), anti-inflammatory and antipyretic drug (Donatien *et al.,* 2011). Powdered leaves are used as an anti-asthmatic and known to have antihistamine and anti-tension properties (De Caluwe *et al.*, 2010). Baobab bark is widely used in traditional medicine as a substitute for quinine in case of fever or as a prophylactic (De Caluwe *et al*., 2010).

Despite the large array of information about the therapeutical, neutraceutical, cosmoteutical and ethnomedicinal uses of *A. digitata,* there is paucity of scientific information about its antimalarial activity. The present research therefore sought to investigate the effect of methanol and flavonoid-rich extracts of the leaf of *A. digitata* on the Plasmodium parasite.

1.1 Study Plant: *Adansonia digitata*

A. digitata (baobab tree in both English and French) is a characteristic plant of the Sahelian region and belongs to the Bombaceae family (De Caluwé *et al*., 2010). The name commemorates the French botanist Michel Adanson (1727- 1806). Linneaus dedicated the genus and species to him; 'digitata' means hand shaped, referring to the shape of the leaf. Common names for the baobab include dead-rat tree (from the appearance of the fruits), monkey-bread tree (the soft, dry fruit is edible), upside-down tree (the sparse branches resemble roots) and cream of tartar tree (cream of tartar). Baobab, a plant which derived its scientific name "*A. digitata*" from the French explorer and botanist, Michel Adanson (1727-1806). He officially discovered it in 1749 on the island of Sor in Senegal (Michel, 2015). "*Digitata*" refers to the digits of the hand. The Baobab's branches and leaves are akin to a hand. It is a traditional food plant in Africa that is high in antioxidants, and has three times the vitamin C of an orange (The Independent, 2015).

The plant is a very massive tree with a very large trunk (up to 10 m diameter) which can grow up to 25 m in height and may live for hundreds of years. It is widespread throughout the hot and drier regions of tropical Africa (Donatien *et al.,* 2011; De Caluwe *et al.*, 2010).

A. digitata is a large, round canopied tree with a swollen trunk, about 10-25 m in height (Gebauer *et al.,* 2002), often with a bole of 3-10 m bark is soft, smooth, fibrous, reddish-brown, greyish-brown or purplish-grey (Gebauer *et al.,* 2002); bark of leaf-bearing branches is normally ashy on the last node; a green layer below the outer, waxy layer of the bark, presumably to assist in photosynthesis when the tree has shed its leaves.

The thick, fibrous bark is remarkably fire resistant, and even if the interior is completely burnt out, the tree continues to live. Re-growth after fire results in a thickened, uneven integument that gives the tree its gnarled appearance resembling an elephant's skin but that

serves as added protection against fire. The fruit of the baobab tree hangs singly on long stalks with an ovoid, woody and indehiscent shell 20 to 30 cm long and up to 10 cm in diameter (Nnam and Obiakor, 2003), embedded in a whitish powdery pulp, have little or no endosperm. Leaves alternate, digitately 3- to 9-foliate; leaflets oblong to ovate, 5-15 x 3-7 cm, lower leaflets being the smallest and terminal leaflet the largest; leaflets dark green, with short, soft hairs; lateral veins looping; apex and base tapering; margin entire; petiolules absent or almost so; petiole up to 12 cm long (Orwa *et al.*, 2009). The ripe fruit pulp appears as naturally dehydrated, powdery, whitish coloured and with a slightly acidulous taste (Vertuani *et al.,* 2002).

Figure 1.0   *A. digitata* **tree plant**

Source: *Wikipedia*

Figure 1.1   *A. digitata* **leaves**

Source: *Wikipedia*

1.1.1 Ecology

The plant is widespread throughout the hot and drier regions of tropical Africa (Donatien *et al.,* 2011). The tree is characteristic of thorn woodlands of the African savannahs, which are characterized by low altitudes with 4-10 dry months a year split into 1 or 2 periods. *A. digitata* is resistant to fire, termite and drought, and prefers a high watertable. It occurs as isolated individuals or grouped in clumps irrespective of soil type. It is not found in areas of deep sand, presumably because it is unable to obtain sufficient anchorage and moisture. It is very sensitive to water logging and frost. All *A. digitata* locations can be described as arid and semi-arid, with not more than a day frost per year.

1.1.2 Widespread Use

Baobab tree has multi-purpose uses and every part of the plant is reported to be useful (Donatien *et al.,* 2011). Its leaves are used in the preparation of soup and they can also be fermented and used as a flavouring agent, or roasted and eaten as snacks (Donatien *et al.,* 2011). The flower is eaten raw, the seeds also provide flour, which is very rich in vitamin B and protein, and it is also used as baby food. The fruit pulp obtained from the seed provides a refreshing drink when dissolve in water or milk (Donatien *et al.,* 2011). The spongy and soft nature of the tree makes it to store water, often chewed by human and animals during extreme scarcity of water.

The bark of the young baobab tree is used in making fishing nets, baskets, light canoes, trays, mats and clothes (Rabi'u and Murtala, 2013; Tukur, 2010). The leaves of the baobab tree are a staple food source for rural population in many parts of Africa especially the central part of the continent (Gebauer *et al.,* 2002; Tukur, 2010). Young leaves are widely used, cooked as spinach,

and frequently dried, often powdered and used for sources over porridges, thick gruels of grains or boiled rice. The pulp serves as a fermenting agent in local brewing or as a substitute for tartar in baking. The husk of the fruit is used in making dishes, vessels also as fuel. The roots also provide a very important ingredient for dyes, the ash obtained from burning the tree is used in soap making, and as fertilizer. The long-fibred wood is suitable for firewood. The shell and seeds are also used for fuel, which potters use to smooth earthenware necklaces before firing. It is also used for making gum or resin as glue can be made by mixing flower pollen with water.

1.1.3 Ethnomedicinal Uses of *A. digitata*

Various medicinal uses were discovered from the Baobab tree. The bark of the tree is used in the treatment of fever; infections; wound disinfections; toothache etc. The leaves also are used in the treatment of guinea worm sores, insect's bites, kidney and bladder disorders, diarrhea, ulcers, fatigue, cough, asthma etc (Donatien *et al.,* 2011). The fruit pulp also provide good medicine for malaria, small fox, dysentery and general fatigue for children while the seeds are use in curing diseases like dental disorders. The roots of the tree (*A. digitata*) are used in the treatment of malaria as well (Donatien *et al.,* 2011).

Baobab fruit pulp has a well-documented antioxidant capability, a result of its high natural vitamin C content (Blomhoff *et al.,* 2010; Brady, 2011). Antioxidants could help prevent oxidative stress related diseases such as cancer, aging, inflammation and cardio- vascular diseases as they may eliminate free radicals which contribute to these chronic diseases (Donatien *et al.,* 2011; Blomhoff *et al.,* 2010).

Baobab leaves, bark, pulp and seeds are used as food and for multiple medicinal purposes in many parts of Africa (Diop *et al.,* 2005). Baobab bark treats fever associated with illness

(Wickens and Lowe, 2008; Brady, 2011). Baobab fruit pulp has also been shown to lower elevated body temperature without affecting normal body temperature (Donatien *et al.,* 2011). It is also used in cosmetic treatment; an infusion of roots is used in Zimbabwe to bathe babies to promote smooth skin (De Caluwe *et al.,* 2010). Seed oil is used to treat skin complaints (Sidibé and Williams, 2002). Baobab fruit pulp has traditionally been used as an immunostimulant (Al-Qarawi *et al.,* 2003), anti-inflammatory, analgesic, antipyretic, febrifuge and astringent in the treatment of diarrhoea and dysentery (Donatien *et al.,* 2011) and to promote perspiration (Sidibe and Williams, 2002).

The aqueous extract of baobab fruit pulp exhibited significant hepatoprotective activity and, as a consequence, the consumption of the pulp may play an important part in human resistance to liver damage in areas where baobab is consumed (Al-Qarawi *et al.,* 2003). Oil extracted from seeds is used for inflamed gums and to ease diseased teeth (Sidibe and Williams, 2002). Powdered leaves are used as a tonic and an anti-asthmatic and known to have antihistamine and anti-tension properties. The leaves are also used to treat insect bites, Guinea worm and internal pains, dysentery, diseases of the urinary tract, opthalmia and otitis (Sidibe and Williams, 2002).

Baobab leaves are used medicinally as a diaphoretic, an astringent, an expectorant and as a prophylactic against fever (Donatien *et al.,* 2011). Leaves are used to treat kidney and bladder diseases, asthma, general fatigue, diarrhoea, inflammations, insect bites and guinea worm (Donatien *et al.,* 2011). The widest use in tradition medicine comes from the baobab bark as a substitute for quinine in case of fever or as a prophylactic. A decoction of the bark deteriorates rapidly due to the mucilaginous substances present (Sidibe and Williams, 2002).

Moreover, the bark contains a white, semi-fluid gum that can be obtained from bark wounds and is used for cleansing sores (Donatien *et al.,* 2011). They contain the alkaloid "adansonin", which has a strophanthus-like action (Donatien *et al.,* 2011).

In summary, *A.digitata* has been investigated for its anti-inflammatory properties (De Caluwe *et al.,* 2010), and its activity attributed to the presence of sterols, saponins and triterpenes in the aqueous extract (Donatien *et al.,* 2011; Brady, 2011). Its anti-pyretic activity (Donatien *et al.,* 2011), analgesic effect (Donatien *et al.,* 2011; Masola *et al.,* 2009), antimicrobial activity (Yagoub, 2008), antioxidant property (Vertuani *et al.,* 2002) has been confirmed and thus attributed to the presence of various bioactive ingredients (Chadare *et al.,* 2009).

1.2 Justification

The prevalence of malaria as well as the growing incidence of deaths resulting from the disease coupled with the increase in the resistance of malaria parasite to synthetic drugs has led to the increasing search for alternative treatment strategy. Plants constitute a natural reservoir of phytochemicals with potentials for the treatment/management of many diseases. *A. digitata* has a rich history of ethnobotanical usage in the treatment of a wide range of illnesses including malaria but with paucity of scientific information in this regard. The present study is thus necessary to fill this lacuna.

1.3 Aim and Objectives of Study

1.3.1 Aim

The aim of this study is to investigate the antimalarial activity of *Adansonia digitata* stem bark.

1.3.2 Objectives

The specific objectives of this study are to:

i. prepare the methanol and flavonoid-rich extracts of *A. digitata* stem bark;

ii. assess the antimalarial properties of the methanol and flavonoid-rich extracts of the stem bark on plasmodium berghei-infected mice; and

iii. investigate the effect of the extracts on oxidative stress indices in *Plasmodium berghei-*infected mice.

CHAPTER TWO

2.   LITERATURE REVIEW

2.1   Malaria

Malaria, the most important parasitic disease of man, is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a group of single-celled microorganism). The protozoan belongs to the genus *Plasmodium*. It is a life-threatening blood disease caused by a Plasmodium parasite. The disease is transmitted most commonly by an infected female Anopheles mosquito. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood (WHO, 2014).The parasites travel to the liver where they mature and reproduce. Five species of plasmodium can infect and be spread by humans. Most deaths are caused by *P. falciparum* because *P. vivax*, *P. ovale*, and *P. malariae* generally cause a milder form of malaria. The species *P. knowlesi* rarely causes disease in humans (Caraballo 2014; WHO, 2014).

2.1.1   History

The term malaria originates from Medieval Italian: ***mala aria*** meaning "bad air" the disease was formerly called *ague* or *marsh fever* due to its association with swamps and marshland (Lindemann, 1999). Malaria was once common in most of Europe and North America (Gratz, 2006) where it is no longer endemic (Webb, 2009), though imported cases do occur (Alphonse, 2012).

Hippocrates from studies in Egypt was first to make connection between nearness of stagnant bodies of water and occurrence of fevers (David *et al.,* 2004),which can be traced back

to the beginning of 2700 BC in China, labelling them tertian, quartan, subtertian and quotidian (Sallares and Gomzi, 2001). The Roman Coulumella associated the disease with insects from swamps (Sallares and Gomzi, 2001) and also pioneered the efforts to drain swamps (David *et al.,* 2004). Several regions in ancient Rome were considered at-risk for the disease which then was known as "Roman fever" (Hays, 2005), because of the favourable conditions present for malaria vectors. The presence of stagnant water in these regions of Rome was preferred by mosquitoes for breeding grounds. Irrigated gardens, swamp-like grounds, runoff from agriculture, and drainage problems from road construction led to the increase of standing water (Reiter, 1999).

Scientific studies on malaria made their first significant advance in 1880, when Charles Louis Alphonse Laveran, a French army doctor, observed parasites inside the red blood cells of infected people for the first time (David *et al.,* 2004), this led him to propose that malaria is caused by this organism, the first time a protist was identified as causing disease (Tan & Sung, 2008). Finlay, a Cuban doctor, in 1908, provided strong evidence that mosquitoes were transmitting disease to and from humans (Chernin, 1983). In 1898, Sir Ronald Ross showed that certain mosquito species transmit malaria to birds, he proved mosquito as the vector for malaria in humans and also elucidated the complete life cycle of malaria parasite in mosquitoes.

2.1.2 Prevalence

Malaria is presently endemic and it is a major threat to public health in various parts of the world, around the equator and areas such as parts of Asia, Latin America, Middle East, Eastern Europe, Pacific and much of Africa, where it is largely prevalent causing 85-90% of malaria fatalities especially in the Sub Saharan Africa (Layne, 2007). The prevalence of malaria in the tropical and subtropical regions have been attributed to rainfall, consistent high

temperatures and high humidity as well as the presence of stagnant waters in which mosquito larvae readily mature, thus providing a favourable environment for the continuous breeding of this vector (Jamieson *et al.*, 2006).

In 2010, World Health Organisation estimates stated that 219 million cases of malaria resulting in 660,000 deaths occurred (Nadjm and Behrens, 2012; Olupot-Olupot and Maitland, 2013). Others have estimated the number of cases at between 350 and 550 million for falciparum malaria (Murray *et al.*, 2012). Death toll as a result of this disease rose from about 1.0 million deaths in 1990 (Layne, 2007) to about 1.24 million in 2010 (Lozano *et al*., 2012) with majority of the cases (65%) occurring in children under 15 years old (Lozano *et al*., 2012). In the sub Saharan Africa, where malaria is endemic, about 125 million pregnant women are at risk of infection each year and an estimated infant death of about 200,000 have been attributed to maternal malaria (Hartman *et al., 2012*). There are about 10,000 malaria cases per year in Western Europe, and 1300–1500 in the United State (Taylor *et al*., 2012). In 2012, of the 207million cases of malaria and an estimated death of between 473,000 and 789,000 people, Africa children were the hardest hit (WHO, 2014).

The World Health Organisation reported that there were 198 million cases of malaria worldwide in 2013 (GBD, 2013; WHO, 2014) which resulted in an estimated 584,000 to 855,000 deaths, the majority (90%) of which occurred in Africa (W.H.O., 2014). Malaria is presently endemic in a broad band around the equator, in areas of the Americas, many parts of Asia, and much of Africa; in Sub-Saharan Africa, 85–90% of malaria fatalities occur (Provost, 2011).

The prevalence of malaria in the tropical and subtropical regions can be attributed to rainfall, consistent high temperatures and high humidity, along with stagnant waters in which

mosquito larvae readily mature, providing them with the environment they need for continuous breeding (Abeku, 2007). In some parts of the world, malaria is more common in rural areas than in cities (Machault *et al.,* 2011), however, malaria in Africa is present in both rural and urban areas with the risk being very low in larger cities (Harper and Armelagos, 2011).
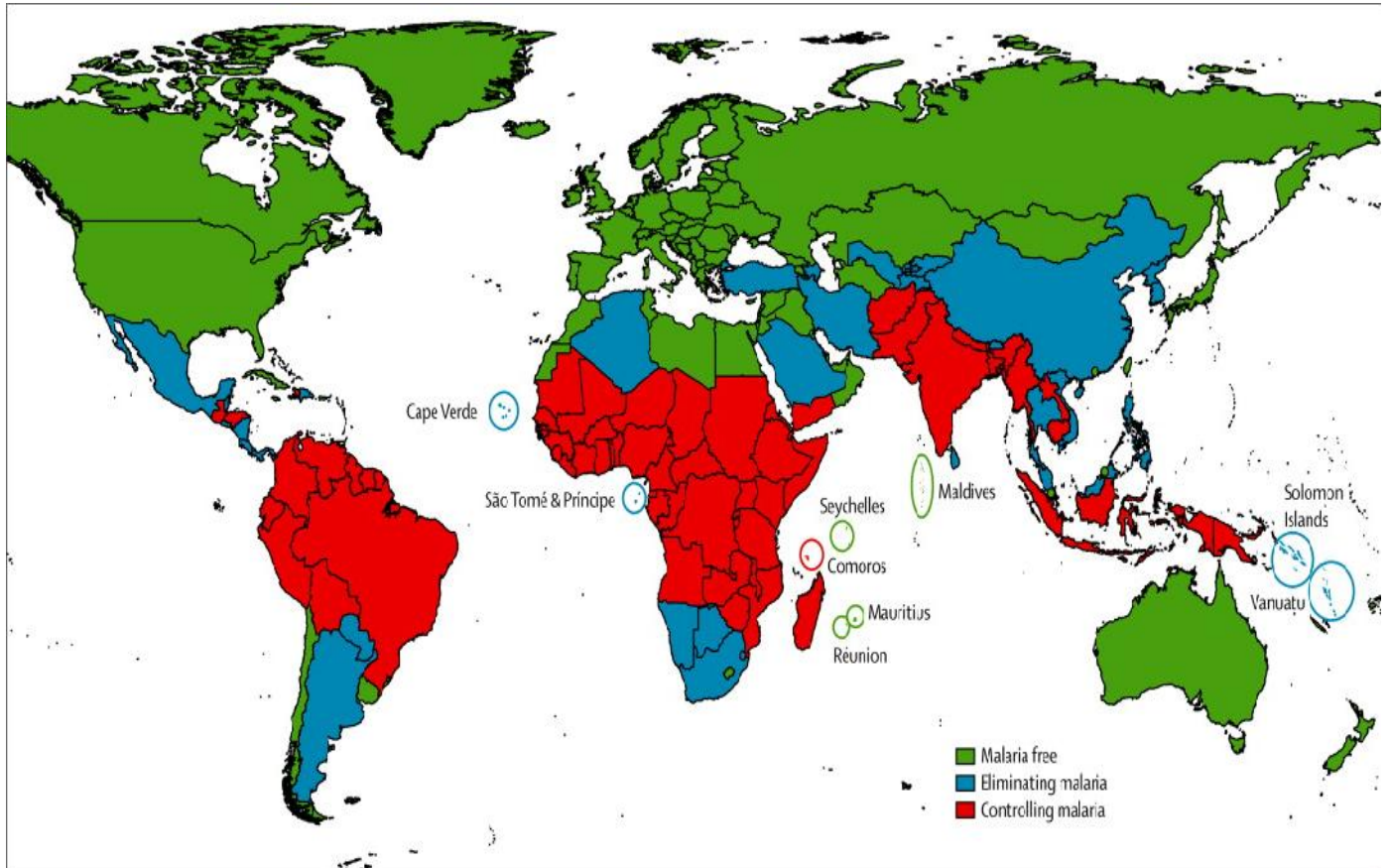
Figure 2.1: **Map showing the prevalence of Malaria in the world**

Source: Wikipedia

2.1.3   Causes of Malaria

Malaria is caused by a small living organism, called a parasite, which infects a person's red blood cells. The malaria parasites belong to the genus *Plasmodium.* The disease is transmitted most commonly by an infected female *Anopheles* mosquito. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood (WHO, 2014). Five species of the parasite which include *P. falciparum, P malariae, P. ovale, P. vivax, P. knowlesi*, have been identified to cause malaria in humans (Mueller *et al.,* 2007; Collins, 2012) with *P. falciparum* being the most common species associated with most deaths followed by *P. vivax* (Nadjm and Behrens, 2012) while others cause a milder form of malaria.   Although *P. falciparum* traditionally accounts for the majority of deaths (Sarkar *et al.,* 2009), recent evidence suggests that *P. vivax* malaria is associated with potentially life-threatening conditions about as often as with a diagnosis of *P. falciparum* infection (Baird, 2013). *P. vivax* proportionally is more common outside Africa (Arnott *et al.,* 2012). There have been documented human infections with several species of *Plasmodium* from higher apes; however, except for *P. knowlesi* (a zoonotic species that causes malaria in macaques-(Collins, 2012)-there are mostly limited public health importance (Collins & Barnwell, 2009). *P. knowlesi* rarely causes disease in humans (Caraballo, 2014; WHO, 2014).

2.1.4   Malaria Vector

Figure 2.2: **Malaria vector, female anopheles mosquito**

Source: *Wikipedia*

Kingdom   Animalia

Phylum   Arthropoda

Class   Insecta

Order   Diptera

Family   Culicidae

Tribe   Anophelini

Genus   Anopheles (Meigen, 1818)

Anopheles is a genus of mosquito that was first and described by J.W. Meigen in 1818 (Nadjm& Behrens, 2012). While over 100 of the 460 species recognized can transmit human malaria, only 30-40 commonly transmit parasites of the genus *Plasmodium,* which cause malaria in humans in endemic areas. *Anopheles gambiae* is best known for its predominant role in the transmission of *P. falciparum,* the most dangerous malaria parasite species (to humans). Anopheles mosquitoes go through four stages in their life cycle with the egg, larva and pupa stage being aquatic for 5-14 days, depending on the species and the temperature, the fourth stage being the imago stage.

Female *Anopheles,* like the males, feed on sugar sources for energy, but usually require a blood meal for the development of eggs. The female *Anopheles* mosquito acts as a malaria vector in the adult stage in which it can live up to a month but probably do not live more than two weeks in nature. *Anopheles* mosquitoes carrying malaria parasites are significantly more

attracted to human breath and odours than uninfected mosquitoes (GBD, 2013). Most *Anopheles* mosquitoes are crepuscular (active at dusk or dawn) or nocturnal (active at night). Some feed indoors (endophagic), while others feed outdoors (exophagic).

2.1.5   Malaria Parasite and Its Life Cycle

Kingdom   Chromalveolata

Superphylum   Alveolata

Phylum   Apicomplexa

Class   Aconoidasida

Order   Haemosporida

Family   Plasmodidae

Genus   Plasmodium   (Marchoafava & Celli,1885)

The malaria parasite is a mosquito- transmitted protozoan, which belongs to a large genus of parasitic protozoa named *Plasmodium (*Phylum *Apicomplexa).*   Plasmodia are sporozoan parasites of red blood cells transmitted to animals (mammals, birds, reptiles) by mosquito's bites. The five species of this genus which are the main causes of malaria in humans are *P. falciparum, P. malariae, P. ovale, P. vivax, P. knowlesi (*Mueller *et al.,* 2007; Collins, 2012*).* In sub- Saharan Africa, most malaria episodes are caused by *P. falciparum*, which is the agent of the most severe and fatal malaria disease followed by *P. vivax* (Nadjm and Behrens, 2012) especially outside Africa and *P. knowlesi*, common in Southeast Asia, where it is dangerous. Transmission of the

plasmodium parasite is mainly from person to person through the bite of a female Anopheles mosquito. Rarely transmission can be through accidents, such as transfusion, inoculation of infected blood from one person to another, or transfer through the placenta from an infected mother to her unborn child.

The malaria parasite has a complex life cycle involving two host: a mosquito vector and a vertebrate host. The life cycles of all plasmodium species transmitted to humans have adapted to man over the years. It involves a sequence of different stages in both the vector and the host. The sexual stages develop in the mosquito's midgut where the gametocytes develop into gametes which fertilize each other. Motile zygotes develop through fertilization and escape the gut and thus grow into new sporozoites which migrate into the mosquito's salivary glands, a site where they are readily injected into the mosquito's next host. When infected mosquitoes bite humans, the sporozoites are injected into the host's blood and thus undergoes stages which involves; latent hypnozoites; merosomes and merozoites which infect the red cells (erythrocytes) of the blood; trophozoites which grow in the red cells, and schizonts which divide there, producing more merozoites which leave to infect more red cells.

The sexual cycle of malaria parasites begins when a particular species of female Anopheles mosquito (the definitive host) feeds on an infected person. Male malaria parasites (microgametocytes) in the infected person's blood, produces flagella which swim towards the female malaria parasite (macrogametocytes), thus fertilizing it. The zygote squeezes its way between the cells of the stomach forming oocyst. The oocyst ruptures to release a spindle – shaped sporozoite which eventually makes its way into the mosquito salivary gland. Mosquito

parasite life cycle is often completed between 7-21 days depending on the specie, ambient temperature and humidity.
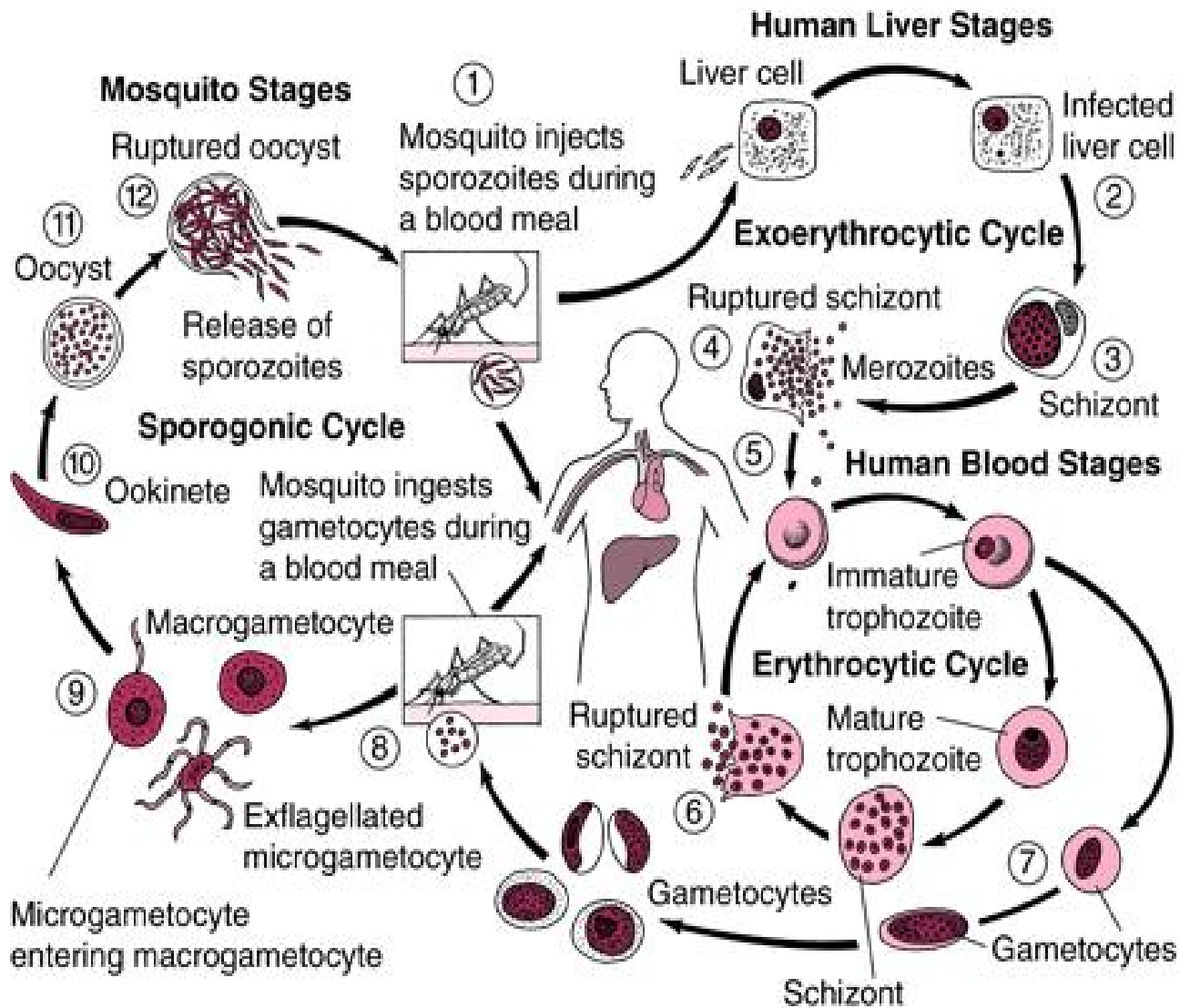
Figure 2.3:   Life cycle of the malaria parasite

Source: Wikipedia

Biting of humans (secondary host) by an infected female anopheles mosquito leads to the introduction of sporozoites with the saliva that the mosquito uses as an anticoagulant into the body system. This anticoagulant prevents the blood from clotting in the mosquito's very small, tube-like proboscis or mouth parts. Once inside human beings, the sporozoites moves quickly to invade the liver cells where they reproduce asexually. The liver schizont, finally bursts (Schlangehof-Lawlor, 2008), releasing thousands of merozoites into the bloodstream, which quickly adhere to and enter red blood cells while other merozoites develop into immature gametocytes which are the precursors of male and female gametes. On entering a red blood cell, the malaria parasite starts growing, using the contents of the cell as food, and becomes ringed form, trophozoites. The life cycle of malaria parasite which takes place in two hosts results in symptoms which are consequences of infection of human erythrocytes.

2.1.6   Symptoms of Malaria

The clinical manifestations of malaria are dependent on the previous immune status of the host. The signs and symptoms of malaria typically begin 8–25 days following infection (Fairhurst and Wellems, 2010). Headache, fever, shivering, joint pain, vomiting, hemolytic anemia, jaundice, hemoglobin in the urine, convulsions and retinal damage are some of the symptoms of malaria (Beare *et al*., 2006). According to World Health Organisation, Malaria can be classified into either "severe" or "uncomplicated" (Nadjm and Behrens, 2012).   Decreased consciousness, significant weakness, inability to feed, two or more convulsions, low blood pressure, breathing problems, circulatory shock, kidney failure or hemoglobin in the urine , pulmonary Oedema are some of the complications of malaria .

2.1.7 Diagnosis

Malaria is usually confirmed by microscopic examination of blood films or by antigen based rapid diagnostic tests (RTD) (Abba *et al.,* 2011; Kattenberg *et al.,* 2011) with the most effective method being the microscopy (WHO, 2010) which is commonly used to detect malaria parasite (Wilson, 2012). The sensitivity of blood films ranges from 75-90% in optimum conditions to as low as 50%. Rapid diagnostic tests are often more accurate than blood films at predicting the presence of malaria parasites, but they are widely variable in diagnostic sensitivity and specificity and give only qualitative results (Wilson, 2012).

2.1.8 Treatment

Malaria is treated with antimalarial medications which depend on the type and severity of the disease. Uncomplicated malaria may be treated with oral medications. Artemisinin-combination therapy (ACT) which involves the use of artemisinins in combination with other antimalarials have been the most effective treatment for *P. falciparum* infection, it decreases resistance to any drug component (Kokwaro, 2009). Artemisinin-combination therapy is about 90% effective when used to treat uncomplicated malaria (Howitt *et al.,* 2012). Intravenous use of antimalarial drugs has been recommended for the treatment of severe malaria for which artesunate proves to be more active than quinine.

However, due to the increasing resistance being developed by malaria parasite to the standard antimalarial drugs, the world appears to be rediscovering the importance of traditional medicines in Alternative Medicines, or Complementary and Alternative Medicine (CAM).

2.2 Oxidative Stress in Malaria

The role of oxidative stress during malaria infection is still unclear. Recent studies suggest that the generation of reactive oxygen and nitrogen species (ROS and RNS) associated with oxidative stress, plays a crucial role in the development of systemic complications caused by malaria. Malaria infection induces the generation of hydroxyl radicals (OH•) in the liver, which most probably is the main reason for the induction of oxidative stress and apoptosis (Guha *et al*., 2006). Additionally, Atamna *et al*., (1993), observed that erythrocytes infected with *P. falciparum* produced OH• radicals and H_2O_2 about twice as much compared to normal erythrocytes.

A potential source of free radical production in this disease is the host's hemoglobin molecule, since the parasite uses this molecule as a source of amino acids for its own nutrition during the erythrocytic stage of the disease, resulting in the liberation of large amounts of circulating heme. By having Fe^{2+}-associated groups, these heme groups are able to induce intravascular oxidative stress, causing changes in erythrocytes and endothelial cells and facilitating the internalization of the parasite in tissues such as the liver and brain (Kumar and Bandyopadhyay, 2005). Additionally, host-parasite interactions are quite complex and promote constant changes in the delicate balance between pro-oxidant and antioxidant molecules since the host and parasite are capable of producing both. Nevertheless, even anti-malarial drug therapy constitutes a source of oxidation, as many drugs such as chloroquine, primaquine and derivatives of artemisinin are inducers of free radical production (Bolchoz *et al.,* 2002; Haynes and Krishna, 2004).
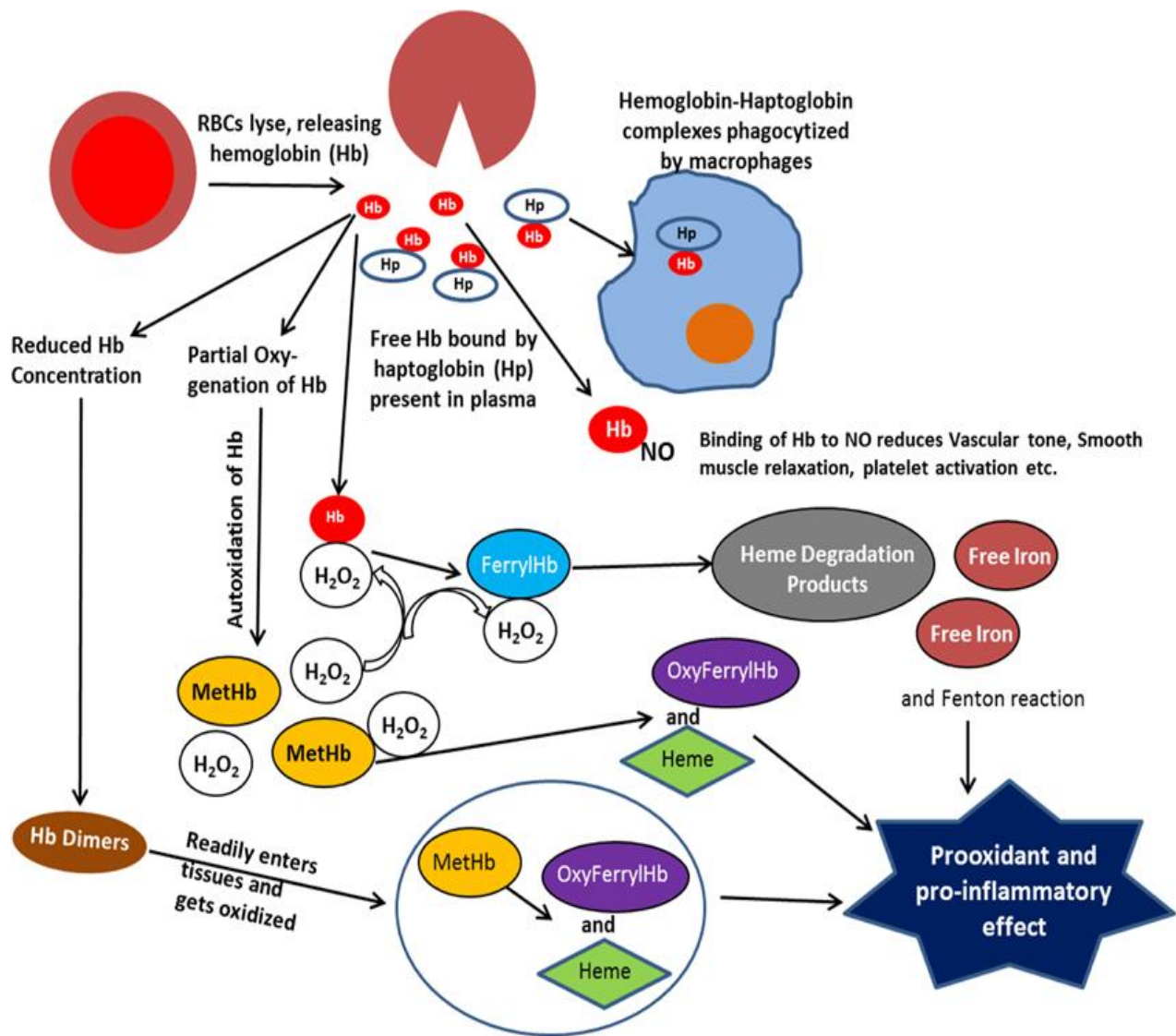
Figure 2.4:   Oxidative stress in malaria

Source: Wikipedia

Oxidative stress is commonly observed to arise from five sources during malaria pathophysiology:

1. Inflammatory process initiated in the host in response to infection;

2. transition metal catalysis, since in feeding on hemoglobin, the parasite releases significant amounts of free iron;

3. the occurrence of ischemia-reperfusion syndrome, resulting from cytoadherence processes and anemia triggered by infection;

4. direct reactive species production by the parasite;

5. action of antimalarial drugs (Sandro *et al.*, 2012).

2.2.1 Host Immune Response as a Source of Oxidative Stress

In response to infection caused by Plasmodium parasites, the natural host defense mechanism is activated with involvement of phagocytes (macrophages and neutrophils). These, in turn, generate large amounts of reactive oxygen species (ROS) and reactive nitrogen specie (RNS), causing an imbalance between the formation of oxidizing species and the activity of antioxidants. This imbalance is what triggers oxidative stress, which is an important mechanism of human hosts in response to infections and, in the case of malaria, can lead to the death of the parasites (Sandro *et al.,* 2012).

It has been shown that oxidative stress could be related to a protective role in malaria patients, as possible agents capable of destroying the Plasmodium. Thus, H_2O_2 and $O_2^{\bullet-}$ can operate independently as cytotoxic agents or form other toxic molecules, including radical OH•, hypochlorous acid (HOCl) and peroxynitrite (ONOO−) in the presence of NO (Guha *et al.,* 2006). The increased production of reactive oxygen species (ROS) by phagocytes, as part of the

host defense, is a primary event. ROS generated by macrophages are known as non-specific effector molecules in the host's defense arsenal, which can contribute to oxidative damage in the parasite as well as parasitized erythrocytes (Sandro *et al.,* 2012), once ROS are able to diffuse through the membrane of red blood cells (Das and Nanda, 2009). The inflammatory process mediated by effector immune response mechanisms is required as the homeostasis of the organism is modified. However, in some cases, such as in malaria, these mechanisms are not sufficient to eliminate some Plasmodium species and to some extent, may act in a manner that further harms the host cell (Sandro *et al.,* 2012).

2.2.2   Hemolysis as an Oxidative Stress Induction Factor in Malaria

During the erythrocytic phase of malaria, red blood cell lysis and the release of hemozoin occurs, which consists primarily of ferriprotoporphyrin IX dimers and monomers (FP) and methemoglobin in plasmodial proteins. Free heme is a powerful free radical generator, which can cause serious molecular damage to both host and parasite; the heme group contains Fe^{2+} atoms that can catalyze Fenton and Haber-Weiss reactions, generating free radicals. This is why certain drugs, such as chloroquine, have an active mechanism to prevent hemozoin formation, promoting accumulation of free heme. Thus, chloroquine increases the availability of intracellular heme by disrupting the plasma membrane structure and increasing oxidative stress in Plasmodium (Parikh *et al.,* 2004; Jaramillo *et al., 2005*).

As a result of oxidative stress, lipid peroxidation occurs, promoting functional and structural changes of the plasma membrane that lead to hemolysis. Hemolysis or extensive cell damage can lead to increased concentrations of free heme, causing oxidative stress and

inflammation. Cellular response to hemozoin entails cytokine release (Jaramillo *et al., 2005*; Keller *et al.,* 2006) and generation of reactive oxygen species such as NO (Moore *et al.,* 2004).

2.2.3 Oxidative Stress and the Membrane of Infected-Erythrocyte

During the development of the blood esquizogeny, *P. falciparum* trophozoites increase the viscosity of red blood cells by causing changes in the parasitized cell surface permitting its adhesion to the endothelial wall of capillaries, which seems to be a defense mechanism of the parasite, preventing the passage of parasitized red blood cells through the spleen and their consequent destruction (Sandro *et al.,* 2012). Among the changes that take place on the surface of red blood cells is the phenomenon of lipid peroxidation. In this sense, the parasitized erythrocytes are known to contain large amounts of monohydroxy derivatives of polyenoic fatty acids (OH-PUFA) in their lipids, suggesting the occurrence of lipid peroxidation due to the release of heme iron from non-enzymatic breakdown (Schwarzer *et al.,* 2003).

Additionally, oxidative changes in *P. falciparum*-infected red blood cells seem to be associated with the accelerated aging of these cells and contribute to the development of anemia presented by these subjects (Omodeo-Salè *et al.,* 2003). The development of anemia can promote changes in the circulatory physiology, leading to the existence of moments of hypoxia alternating with the maintenance of tissue oxygena

tion at basal levels, favoring the participation of ischemia and reperfusion syndrome (IRS) responsible for an additional production of free radicals (Halliwell and Gutteridge, 2007).

2.2.4   Oxidative Changes in *Plasmodium*

2.2.4.1 Production of Reactive Species by the Parasite

Besides host ROS/RNS production in response to infection, the parasite itself is capable of producing free radicals, which in turn interfere with the biochemistry of red blood cells and may promote or facilitate the internalization of the parasite in hepatocytes and RBC. Aerobic membrane transport mechanisms are a major source of free radical and ROS/RNS generation in Plasmodium (Sandro *et al.,* 2012).

2.2.4.2   Antioxidant Defense Mechanisms in the Parasite

Plasmodium parasites are subjected to high levels of oxidative stress during development in host cells, so that their ability to defend themselves against this aggression is critical to their survival. As a result, these parasites have developed several antioxidant defense mechanisms.

Additionally, to compensate for the oxidative stress suffered, Plasmodium reduces its own production of reactive oxygen species and adapts new mechanisms to prevent oxidative damage arising from the host. The apicoplast is one such mechanisms; it is a symbiotic intracellular organelle located near the mitochondria which seems to synthesize lipoic acid, a potent antioxidant used by the parasite as a defense. Most probably this organelle was incorporated as an evolutionary adaptation of the parasite, since this organelle is also present as a symbiont in red algae (Toler, 2005).

Moreover, in most Plasmodium cells, the redox homeostasis seems to be based on the synthesis of reduced glutathione and thioredoxin system (Trx)/thioredoxin reductase (TrxR). The reduction of oxidized glutathione (GSSG) can be supported by the high proportion of the TrxR/Trx system in glutathione reductase-deficient cells, which may be important for certain

stages of the parasite (Becker *et al.,* 2004; Kanzok *et al.,* 2000). The glutathione and thioredoxin redox systems represent two powerful means to detoxify reactive oxygen species in *P. falciparum* and they are efficient systems to prevent parasite development inside the host cells (Müller *et al.,* 2001).

Additionally, an enzyme peroxiredoxin associated with chromatin in *P. falciparum* has been identified, which makes use of thioredoxin and glutaredoxin as reducing agents, thereby conferring protection to the parasite against the oxidative insult imposed by the host (Richard *et al.,* 2011). The TrxR, an enzyme involved in the maintenance of redox homeostasis and antioxidant defense, is essential for the erythrocytic stages of *P. falciparum* (Kehr *et al.,* 2010). The disruption of the parasite antioxidant system is a feasible way of interfering with its development during erythrocytic schizogony (Krnajski *et al.,* 2002).

2.2.5   Antimalarial Drugs and Oxidative Stress

Quinine, extracted from the Cinchona genus tree or shrub bark in the tropical region of South America, was among the pioneer antimalarial drugs. Although the active mechanism of quinine is still not understood well, and despite being used for over 100 years, it is commonly believed to interfere with DNA replication of Plasmodium (Sandro *et al.,* 2012). Quinine was one of the first antimalarial drugs widely used to control the disease, but has fallen into disuse owing to emerging parasite strains resistant to the drug. Consequently, its use has been replaced by more effective synthetic drugs derived from the acridine and quinoline structure, such as chloroquine and mefloquine, aimed at inhibiting heme polymerase and preventing the polymerization of heme to hemozoin, thereby causing oxidative-metabolic effects on the

parasite, since iron from the heme group can catalyze reactions that generate free radicals (Wongtrakul *et al.,* 2010) and primaquine, which destroys the gametocytes of malaria parasites.

The pharmacological therapy currently used is based on the susceptibility of the genus Plasmodium to free radicals and oxidants, as well as the interference or inhibition of a metabolic synthesis pathway of a molecule essential to the parasite (Grahame-Smith *et al.,* 2004). In fact, several substances used as antimalarials are pro-oxidants, which are why they have pharmacological power (Sandro *et al.,* 2012). This is the case for chloroquine, primaquine, and artemisinin among others.

The *Artemisia annua* plant (Artemisia) is known to be the most ancient antimalarial treatment, having been used in China for over 2000 years (Sandro *et al.,* 2012). It contains artemisinin, a substance which eliminates the blood-stage parasites more rapidly than any other drug and works well against *P. falciparum* species that are resistant to other drugs. This drug produces free radicals in contact with iron, a common metal in the body, especially within erythrocytes (Grahame-Smith *et al.,* 2004). This mechanism is extremely effective in the destruction process of parasites and causes minimal adverse effects to the host (Sandro *et al.,* 2012).

Treatment with artemisinin can provide rapid recovery and leads to elimination of parasites, but the reappearance of parasitemia is frequent, which can be explained by the low half-life elimination time of the drug (Sandro *et al.,* 2012) and by the decrease of plasma concentrations after repeated doses (Giao *et al.,* 2001). Several studies have demonstrated the involvement of oxidative stress in the mechanism of action of artemisinin (Hartwig *et al.,* 2009; Klonis *et al.,* 2011). Some associations which were tested in treating malaria involved: metalloporphyrins/ artemisinin (Sandro *et al.,* 2012) and antimalarial/ oxidizing reagents that act

synergistically (Sandro *et al.,* 2012*).* It is worth mentioning that metalloporphyrins/ artemisinin effectively act on strains of *P. falciparum* resistant to chloroquine (Sandro *et al.,* 2012).

Moreover, the parasite's ability to express antioxidant proteins is suggested to be one of the resistance mechanisms to antimalarials, since early transcriptional response of genes involved in antioxidant protein expression confers the adaptive capacity to certain antimalarial drugs (Nogueria *et al.,* 2010). Other pro-oxidant treatment strategies include alternative therapies with antifungal agents such as clotrimazole, based on their ability to inhibit hemo-peroxidase with consequent oxidative stress induction (Trivedi *et al.,* 2005).

2.3   Phytochemicals

Phytochemicals are naturally occurring compounds in plants, though not established nutrients, but possess much biological significance. They are responsible for the medicinal effects of plants as well as the colour and organoleptic properties of food. They include:

Saponins -   found in vegetables, peas and herbs, they are glucosides with foaming characteristics. Saponins consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Saponins have a bitter taste. Some saponins are toxic and are known as sapotoxin.

Alkaloids -   chemical compounds found in higher plants. They contain mostly basic nitrogen atoms. Examples include quinine, quinidine etc. they have a wide range of action including antimalarial, vasodilatory as well as antibacterial effect.

Polyphenols - they are chemical components that occur ubiquitously as natural colour pigments responsible for the colour of fruits of plants, characterized by the presence of large multiples of phenol structural units. The number and characteristics of these phenol structures underlie the unique physical, chemical, and biological properties.

Others include terpenes, anthraquinones among others. These phytochemicals perform varieties of functions such as antimicrobial, anticarcinogenic, anti-inflammatory, immune modulation, vasodilatory and most importantly, antioxidant functions. Phytochemicals form the bioactive components of plants known as medicinal plants. They may also be regarded to as secondary plant metabolites.

2.3.1   Medicinal Plants

Medicinal plants refer to those plants possessing bioactive chemical components which have profound effects in the treatment of various diseases. The science of applying biologically active chemicals from medicinal plants in the treatment of diseases is referred to as ethno-pharmacology and it dates back to antiquity. The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, quinine, and opium (Swain, 1968). Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in their working principle.

The use of plants as medicine predates written human history. Many of the herbs and spices used by humans to season food also yield useful medicinal compounds (Lichtermann,

2004; Tapsell *et al.,* 2006). Studies show that in tropical climates where pathogens are the most abundant, recipes are the most highly spiced. Angiosperms (flowering plants) were the original source of most plant medicines. Many of the common weeds that populate human settlements, such as nettle, dandelion and chickweed, have medicinal properties (Stepp *et al*., 2001; Sumner & Judith, 2000). A large amount of archaeological evidence exists which indicates that humans were using medicinal plants during the Paleolithic, approximately 60,000 years ago. Furthermore, animals such as non-human primates, monarch butterflies and sheep are also known to ingest medicinal plants to treat illness (Sumner and Judith, 2000).

The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Fabricant and Fahnswort, 2001).

According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Over the years they have assumed a very central stage in modern civilization as natural source of chemotherapy as well as amongst scientist in search for alternative sources of drugs.

Traditional medicines have been used to treat malaria for thousands of years and are the source of the two main groups; artemisinin and quinine derivatives of modern antimalarial drugs (Kazembe *et al.,* 2012). The increase in the incidence of resistant forms of malaria parasites has led to a gradual loss of faith in modern drugs and confidence as well as an increase in the use of herbs in the treatment of malaria (Graz *et al.,* 2011). Emphasis shifted from locally prepared

decoctions of medicinal plants, which had already been used in the treatment of malaria for centuries, when chloroquine, sulphonamides and the third generation line of treatment, artemisinin combination therapy were introduced. Recent evidence suggests confidence in the once abandoned herbs for the treatment of malaria including the resistant forms of malaria parasites (Avwioro, 2010). Cost consideration, absence of side effects such as itches that occur in chloroquine and ineffectiveness of chloroquine (Graz *et al.,* 2011) and some other anti-malaria drugs have led to widespread use of herbs for the treatment of malaria (Graz *et al.,* 2011).

The Peruvian Cinchona tree was one of the inherited anti-fever herbs that led modern science to the discovery of natural quinine as well as several synthetic quinolines, particularly chloroquine. Other medicinal plants used for treating malaria include *Artermisia annua* (from which artemisinin was obtained), *Enantia chloranta, Carica papaya, Mangifera indica, Psidium guajava, A. digitata among others.*

Free radicals produced by normal biochemical reactions have been implicated in various diseases including malaria. The human body possesses innate defence mechanisms to counter free radicals in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Vitamin C, vitamin E, selenium, - carotene, lycopene, lutein and other carotenoids have been used as supplementary antioxidants. Apart from these, plant secondary metabolites such as flavonoids and terpenoids play important role in the defence against free radicals. Medicinal plant parts are commonly rich in phenolic compounds such as flavonoids, stillbenes, tannins, coumarins, lignans and lignins (Kazembe *et al., 2012*). *A. digitata,* among other plants, has been noted as a good medicinal plant for curing various diseases.

CHAPTER THREE

3.0   MATERIALS AND METHOD

3.1   Materials

3.1.1   Chemicals and Reagents

Chemical and reagents used include sodium dodecyl sulphate (SDS), thiobarbituric acid (TBA), folin-ciocatteau's reagent and tannic acid. They were obtained from British Drug House, Poole, England. The reduced glutathione (GSH), thiobarbituric acid and hydrogen peroxide used are products of Sigma Chemical Company, USA, and were obtained from Evergreen Chemical Institute, Nigeria. All other chemicals were of analytical grade. The water used was glass distilled.

3.1.2   Plant Material

The stem of *Adansonia digitata* (Bombacaceae) was collected from a private farm in Ido-Ekiti, Ekiti State Nigeria. The botanical identification and authentication were carried out by Mr. Adeniyi K. A and Mr. Soyewo L. T. at the Herbarium of the Forestry Research Institute (FRIN) Ibadan, Oyo state, Nigeria where a voucher specimen (No 109806) was deposited.

3.1.3   Experimental Animals

Thirty five male albino mice weighing between 18-20g were obtained from the animal house, Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were acclimatized for two weeks in the animal house and fed *ad libitum* on commercial rat chow and water throughout the period of the experiment.

3.1.4 Parasite-*Plasmodium berghei*

The *Plasmodium berghei* used in the transfection was obtained from the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. A standard inoculum of 1×10^7 of parasitized erythrocytes from a donor mouse in volumes of 0.2ml was used to infect the experimental animals intra-peritoneally.

3.2 Methods

3.2.1 Preparation of Crude Methanol Extract of *Adansonia* digitata Stem Bark

The stem bark peels were air-dried at room temperature to avoid possible degradation or denaturation of their putative compounds. The air-dried stem bark of *Adansonia digitata* was pulverized using an electric blender. The powdered material was stored in a glass container. The blended air-dried stem bark (500 g) was soaked in 2 L of methanol for 72 h at room temperature. The mixture was continually stirred for average of 5 min with a glass rod every 24 h. After 72 h of soaking, the mixture was filtered and the filtrate was concentrated using rotary evaporator at 40°C. The concentrate was heated at 50°C over a water bath to obtain a solvent free extract, which was stored in a refrigerator at 4°C.

3.2.2 Preparation of Flavonoid Extract of *Adansonia digitata* Stem Bark

Exactly 3 g of the crude extract was dissolved in 20 ml of 10% H_2SO4 in a small flask and was hydrolyzed by heating on a water bath for 30min at 100°C. The mixture was placed on ice for 15 min, so as to allow the precipitation of the flavonoids aglycones. The cooled solution was filtered, and the filterate (flavonoids aglycone mixture) was dissolved in 50ml of warm 95% ethanol (50°C).The resulting solution was again filtered into 100ml volumetric flask which was

made up to the mark with 95% ethanol. The filtrate collected was concentrated to dryness using a rotary evaporator.

3.2.3 Animal Treatment and Transfection

Prophylactic test: Estimation of the prophylatic effects of the crude methanol extract and the flavonoid extract of *Adansonia digitata* stem bark was carried out according to the method described by Peters (1967).

3.2.3.1 Grouping and Treatment of Experimental Animals

The mice were divided into seven groups of five animals each.

Group I: Mice in this group received by oral gavage, 1 ml/kg of the vehicle (5% v/v tween 80) only once daily for five consecutive days and served as the control.

Group II: Animals were administered chloroquine (5 mg/kg b.wt) by oral gavage once daily for five consecutive days.

Group III: Animals were pretreated with artemether-lumfantrine combination (5 mg/kg b.wt) by oral gavage once daily for five consecutive days.

Group IV: Animals were pretreated with 200 mg/kg b.wt of methanol extract of *Adansonia digitata* (ADME) by oral gavage once daily for five consecutive days.

Group V: Animals were pretreated with 400 mg/kg b.wt of methanol extract of *Adansonia digitata* (ADME) by oral gavage once daily for five consecutive days.

Group VI: Animals were pretreated with 200 mg/kg b.wt of flavonoid rich extract of *Adansonia digitata* (ADFE) by oral gavage once daily for five consecutive days.

Group VI: Animals were pretreated with 400 mg/kg b.wt of flavonoid extract of *Adansonia digitata* (ADFE) by oral gavage once daily for five consecutive days.

After five days of treatment, the mice were transfected intraperitonially with an inoculums size of 1x 10^7 of chloroquine sensitive strain of *plasmodium berghei* infected erythrocytes.

3.2.4 Quantification of Parasitemia and Packed Cell Volume Quantification

After 72 h of transfection, blood samples were collected from the mice tails and smeared on to microscope slides to make both the thick and thin film. The blood films were first fixed in 100% methanol and then stained with Giemsa prepared with buffered water (pH 7.2). Parasitemia was examined microscopically (using x 100 immersion oil objective). Slides were collected for five consecutive days. The packed cell volume (PCV) was determined on day of infection and day five by the microhematocrit method. Percentage parasitemia and percentage clearance/chemosupression were estimated.

%Parasitemia= (Total number of parasitized cells/Total number of cell) x 100%

%Clearance/Chemosupression= [(Negative control parasitemia) – (Parasitamia with drug)]/Negative control parasitemia.

3.2.5 Sacrifice and Collection of Organ from Experimental Animals

Animals were anaesthetized with diethyl ether, dissected and the livers were removed immediately, rinsed in ice-cold 0.9% NaCl solution, blotted with filter paper and weighed.

3.2.6   Preparation of Tissue Homogenate

Livers of rats were minced with scissors in 4 volumes of phosphate buffer saline, pH 7.4 and homogenized in a tefflon homogenizer. The homogenates were later centrifuged at 10000 g for 15 minutes at $4^{o}C$ and the supernatants were collected and stored at $4^{°}C$ until needed for the biochemical assays. The clear supernatant was used to evaluate the extent of membrane lipid peroxidation (LPO) and level of reduced glutathione (GSH).

3.2.7   Estimation of Reduced Glutathione (GSH) Level

The method of Beutler *et al.,* (1963) was followed in estimating the level of reduced glutathione (GSH).

Principle

The reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable (yellow) colour when 5 , 5 – dithiobis - (2-nitrobenzoic acid, DTNB) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman reagent with the reduced glutathione, 2-nitro-5-thiobenzoic acid possesses a molar absorption at 412nm. Reduced glutathione is proportional to the absorbance at 412 nm.

Assay Procedure

The liver homogenate (0.2 ml) was added to 1.8ml of distilled water and 3ml of the precipitating solution (4% sulphosalicylic acid) was gently introduced into the mixture. The mixture was then allowed to stand for approximately 5 minutes and then filtered. At the end of

the fifth minute, 1ml of filtrate was added to 4ml of 0.1M phosphate buffer (pH 7.4). Finally 4.5ml of the Ellman reagent was added.

A blank was prepared with 4ml of 0.1M phosphate buffer, 1ml of diluted precipitating solution (3parts to 2 parts of distilled water) and 4.5ml of the Ellman reagent. The absorbance was measured at 412nm. GSH was proportional to the absorbance at that wavelength and the estimate was obtained from a GSH standard curve.

3.2.8   Assessment of Lipid Peroxidation

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale (1990).

Principle

Under acidic condition, malondialdehyde (MDA) produced from the peroxidation of fatty acid membranes and food products react with the chromogenic reagent, 2-thiobarbituric acid (TBA) to yield a pink coloured complex with maximum absorbance at 532nm and fluorescence at 553nm. The pink chromophore is readily extractable into organic solvents such as butanol.

Assay Procedure

An aliquot of 0.4ml of liver homogenate was mixed with 1.6ml of Tris-KCl buffer to which 0.5ml of 30% TCA was added. Then 0.5ml of 0.75% TBA was added and placed in a water bath for 45 minutes at 80°C. The mixture was then cooled in ice and centrifuged at $3000g$. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532nm. Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56×10^{5} $M^{-1}Cm^{-1}$.

MDA (units/mg protein) = Absorbance x volume of mixture

$$\overline{\text{E}_{532nm} \text{ x volume of sample x mg protein}}$$

Statistical Analysis

Results were expressed as mean ± standard error of mean. Data were analyzed using student`s t-test and one-way analysis of variance followed by Newman Keuls post hoc test. Values of P< 0.05 were considered significant.

CHAPTER FOUR

4. RESULTS

4.1: Antimalarial Effect of Methanol and Flavonoid-Rich Extracts of *Adansonia digitata*

The antimalarial effect of methanol and flavonoid-rich extracts of *A. digitata* stem bark on *P. berghei*-infected mice is depicted in table 4.1.

The result revealed that the highest level of the plasmodium parasite was found in the *P. berghei*-transfected, untreated mice. The administration of extracts resulted in significant ($P<0.001$), dose-dependent decreases in parasite counts (especially on the 3rd and 5th day) in the treated groups when compared to the positive control (Table 4.1). Significant ($P<0.001$) decreases in the level of the malarial parasites in the blood were only observed in mice pretreated with 400 mg/kg b.wt of methanol and flavonoid extracts of *A. digitata* stem bark extract after the first day of transfection with the parasites when compared to the control. At this stage, the effect of treatment with 200 mg/kg b.wt of both extracts were not significant ($P>0.05$). At day 3 and 5 after transfection however, significant ($P<0.001$) decreases in the level of parasites were afforded by both methanol and flavonoid extracts at all dosages (200 and 400 mg/kg) evaluated.

The percentage chemosuppression for each of the extracts increased with increasing number of days following transfection when compared to that of the negative control in which there was no suppression. It could be observed that the flavonoid extract which caused 68% and 82% clearance on 5th day at 200 and 400 mg/kg respectively, appear to have higher activity than the methanol extract with 61% and 68% clearance at similar concentrations.

Table 4.1: Prophylatic effects of methanolic and flavonoid-rich extracts of *A. digitata* stem bark on *P. berghei*-infected mice

Day	1		3		5	
	%P	%C	%P	%C	%P	%C
Control	3.75 ± 0.28	-	4.00 ± 0.07	-	5.27 ± 0.19	-
AL 5	$0.68 \pm 0.07^{***}$	81.87 ± 0.00	$0.00 \pm 0.00^{***}$	100.0 ± 0.00	$0.00 \pm 0.00^{***}$	100.0 ± 0.00
CQ 5	$1.00 \pm 0.12^{***}$	73.33 ± 0.00	$0.00 \pm 0.00^{***}$	100.0 ± 0.00	$0.00 \pm 0.00^{***}$	100.0 ± 0.00
ADME 200	3.30 ± 0.16	12.00 ± 0.00	$2.52 \pm 0.35^{***}$	37.00 ± 0.00	$2.07 \pm 0.19^{***}$	60.72 ± 0.00
ADME 400	$2.40 \pm 0.51^{**}$	36.00 ± 0.00	$1.80 \pm 0.15^{***}$	55.00 ± 0.00	$1.67 \pm 0.59^{***}$	68.31 ± 0.00
ADFE 200	3.00 ± 0.08	20.00 ± 0.00	$2.06 \pm 0.29^{***}$	48.50 ± 0.00	$1.67 \pm 0.44^{***}$	68.31 ± 0.00
ADFE 400	$1.90 \pm 0.21^{***}$	49.33 ± 0.00	$1.37 \pm 0.09^{***}$	65.75 ± 0.00	$0.97 \pm 0.07^{***}$	81.59 ± 0.00

Control: *P. berghei*-infected mice which did not receive any treatment except the vehicle (Tween 80, 5% v/v); **AL 5:** 5 mg/kg b.wt Artemether-Lumefantrine; **CQ 5:** 5 mg/kg b.wt Chloroquine; **ADME 200/ADME 400:** 200/400mg/kg b.wt *A. digitata* methanolic extract; **ADFE 200/ADFE 400:** 200/400 mg/kg b.wt *A. digitata* flavonoid extract; %P= percentage parasitemia; %C= percentage clearance.

Results are mean±standard error of mean (SE) of replicate measurements (n=5).Data analysis was done by one-way ANOVA, followed by NeumanKeuls post hoc test ($P < 0.05$ was considered statistically significant).**P< 0.01,*** P< 0.001 compared with control, untreated group.

Furthermore, the antimalarial effect of the extracts compared well, but lower than those of the reference drugs (5 mg/kg chloroquine and 5 mg/kg arthemether-lumefantrine) which caused significant (P<0.001) decreases right from the first day after transfection (73.33±0.00 and 81.87±0.00 respectively) and 100% clearance at days 3 and 5 after transfection.

4.2: Effect of Methanolic and Flavonoid-Rich Extracts of *Adansonia digitata* on Packed Cell Volume of *P. berghei*-infected mice

The effect of methanolic and flavonoid-rich extracts of A*dansonia digitata stem bark* on packed cell volume of *P. berghei*-infected mice is shown in table 4.2. The highest, significant (P<0.001) decrease in the PCV level on treatment last day was observed in *Plasmodium berghei*-transfected, untreated mice when compared to the level on the first day of treatment. Also, there were significant (P<0.001) decreases in PCV levels on the last day of treatment in mice administered 200 mg/kg b.wt of either methanol or flavonoid extracts of *A. digitata* stem bark extract. Treatment with 400 mg/kg b.wt of both extracts completely reversed the decreases caused by the malaria parasites in the packed cell volume of mice as there were no significant (P>0.05) differences in the PCV in both days.

Pretreatments with 5 mg/kg of the reference drugs, chloroquine and arthemether/lumefantrine (coartem) reversed the decreases in the PCV of mice caused by the malaria parasites as there were no significant (P>0.05) differences when compared to the first day of treatment.

Table 4.2: .Effects of methanolic and flavonoid-rich extracts of *A. digitata* stem bark on packed cell volume *P. berghei*-infected mice

Treatment	Day of Infection	Treatment Last day
Control	45.80 ± 2.78	$15.20 \pm 1.77^{***}$
AL 5	48.20 ± 2.44	44.20 ± 2.08
CQ 5	46.00 ± 1.79	47.20 ± 1.77
ADME 200	47.20 ± 1.69	$24.00 \pm 1.92^{***}$
ADME 400	45.00 ± 2.35	37.00 ± 3.1
ADFE 200	43.40 ± 2.09	$25.00 \pm 0.84^{***}$
ADFE 400	47.20 ± 2.42	42.80 ± 2.35

Control: *P. berghei*-infected mice which did not receive any treatment except the vehicle (Tween 80, 5% v/v);**AL 5:** 5 mg/kg b.wt Artemether-Lumefantrine; **CQ 5:** 5 mg/kg b.wt Chloroquine; **ADME 200/ADME 400:** 200/400 mg/kg b.wt*A. digitata* methanolic extract; **ADFE 200/ADFE 400:** 200/400 mg/kg b.wt *A. digitata* flavonoid extract.

Results are mean±standard error of mean (SE) of replicate measurements (n=5) after five days of exposure to treatment. Data analysis was done by student`s t-test ($P< 0.05$ was considered statistically significant).*** < 0.001 compared with the respective value on the *Day of Infection*.

4.3: Effect of Methanolic and Flavonoid-Rich Extracts of *Adansonia digitata* on hepatic GSH level of *P. berghei* infected mice.

The effect of the methanol and flavonoid-rich extracts of *A. digitata* on hepatic reduced glutathione level of *P. berghei*-infected mice is shown in Fig. 4.1.

The effects of the extracts (ADME and ADFE) at both concentrations evaluated (200- and 400 mg/kg b.wt) and the reference drugs were not significantly (P>0.05) different from the positive control. The highest concentration of GSH was observed in mice pretreated with artemeter/lumefantrine combination.

4.4: Effect of Methanolic and Flavonoid-Rich Extracts of *Adansonia digitata* on hepatocytes membrane peroxidation in *P. berghei* infected mice.

The effect of methanolic and flavonoid-rich extracts of A*dansonia digitata* on hepatic membrane lipid peroxidation of *P. berghei*-infected mice is shown in Fig. 4.2.

The result revealed that the highest level of malondialdehyde (MDA) was found in the *P. berghei*-transfected, untreated mice. The administration of 200 and 400 mg/kg of both ADME and ADFE as well as the reference drugs, reversed the effect of *P. berghei* infection on hepatic lipid peroxidation by causing significant(P< 0.001) decreases in malondialdehyde levels in the treated groups when compared to the control group (Figure 4.2).
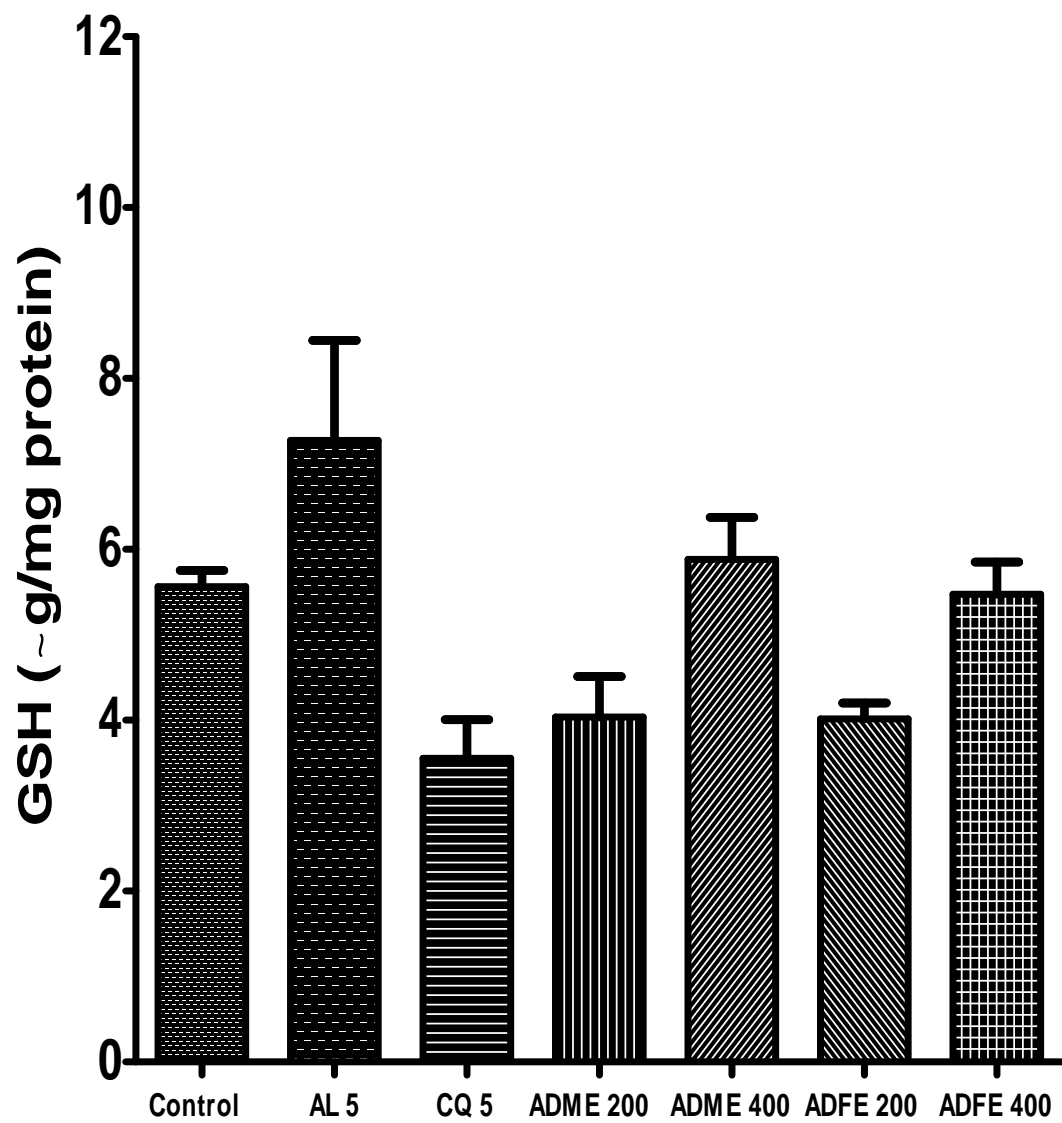
Fig 4.1: Effect of methanol and flavonoid extracts of *Adansonia digitata* on hepatic GSH level of *P. berghei*-infected mice.

Control: *P. berghei*-infected mice which did not receive any treatment except the vehicle (Tween 80, 5% v/v); **AL 5:** 5 mg/kg b.wtArtemether-Lumefantrine; **CQ 5:** 5 mg/kg b.wt Chloroquine; **ADME 200/ADME 400:** 200/400 mg/kg b.wt*A. digitata*methanolic extract; **ADFE 200/ADFE 400:** 200/400 mg/kg b.wt *A. digitata* flavonoid extract.

Results are mean±standard error of mean (SE) of replicate measurements (n=5).Data analysis was done by one-way ANOVA, followed by NeumanKeuls post hoc test ($P < 0.05$ was considered statistically significant).

Fig 4.2: Effect of methanol and flavonoid extracts of *A. digitata* on hepatocytes membrane peroxidation in *P. berghei*-infected mice.

Control: *P. berghei*-infected mice which did not receive any treatment except the vehicle (Tween 80, 5% v/v); **AL 5:** 5 mg/kg b.wt Artemether-Lumefantrine; **CQ 5:** 5 mg/kg b.wt Chloroquine; **ADME 200/ADME 400:** 200/400 mg/kg b.wt *A. digitata* methanolic extract; **ADFE 200/ADFE 400:** 200/400 mg/kg b.wt *A. digitata* flavonoid extract.

Results are mean±standard error of mean (SE) of replicate measurements (n=5). Data analysis was done by one-way ANOVA, followed by NeumanKeuls post hoc test ($P < 0.05$ was considered statistically significant).
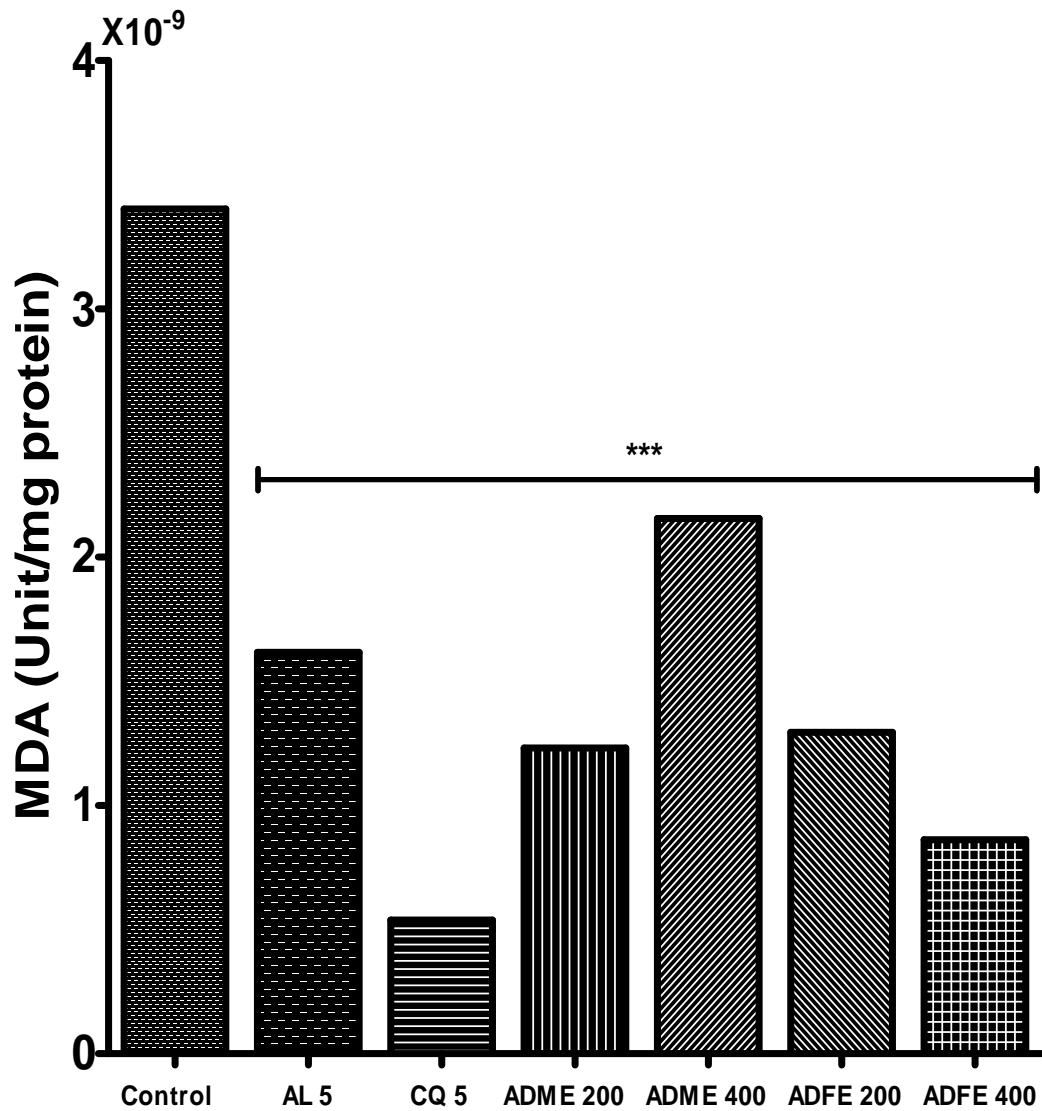
*** $P < 0.001$ compared with control, untreated group.

CHAPTER FIVE

5.0   DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1   Discussion

Medicinal plants have been found to contain phytoconstituents of relevance in phytomedicine (Dahanukar et al., 2002; Somova et al., 2003). Plants have provided active ingredients of medicines for years and are still sources of lead compounds in the development of new therapeutics (Newman, 2008). *A. digitata (*baobab tree in both English and French), is used in the treatment of malaria, fever, among other ailment. Anti-plasmodial effect of medicinal plant substances have been shown to be caused by alkaloids, terpenes and flavonoids (Milliken, 1997; Christensen, 2001).The documented presence of secondary metabolites such as tannins, alkaloids and flavonoids, in *A. digitata*, may be responsible for anti-plasmodium activity of plant (Philipson,1990; Milliken, 1997). *Plasmodium berghei* parasite is used in predicting treatment outcomes of any suspected antimalarial agent (Agbedahunsi, 2000; Adzu and Salawu 2009), due to its high sensitivity to chloroquine making it the appropriate parasite for this study (Peter, 1998; David *et al.,* 2004). *Plasmodium berghei* has been used in studying the activity of potential antimalarials in mice (Pedronic *et al*., 2006). It produces diseases similar to those of human plasmodium infection (Kumar *et al.,* 2006; Peter, 1998).Substances that reduce parasite multiplication (anti-plasmodial effect) in the host were considered to possess antimalarial activity (Ryley and Peters, 1970).

The observed significant (P<0.001) decreases in the level of the malarial parasites in the blood of the mice pretreated with 400 mg/kg b.wt of methanol and flavonoid extracts of *A. digitata* stem bark after the first day of transfection with the parasite, suggest that *A. digitata* possess significant prophylactic effect against malaria infection in *P. berghei*-infected mice..This

justifies the ethnomedicinal use of the plant as anti-malarial agent in sub-Saharan Africa (Sidibe and Williams, 2002; De Caluwe *et al*., 2010). In line with the submission of Kiseko *et al.* (2000) on the effect of standard antimalarial drugs on mice infected with *P. berghei*, chloroquine and arthemeter/lumefantrine used in this study exerted 100% suppression at 5mg/kg body weight (Table 4.1). The dose-dependent nature of the anti-malarial effect of both ADME and ADFE is evident by the observation that the chemosupression offered increases with increasing concentration of the extracts, with the 400mg/kg b.wt of each proving to be more effective than the 200mg/kg b.wt. Consequently, the highest chemosuppression of 68.31% and 81.59% were recorded for mice placed on 400 mg/kg b.wt of ADME and ADFE on the third day of transfection. It is also noteworthy that *A. digitata* flavonoid extract proved more effective of both extracts with percentage parasitemia and chemosuppression values being closest to those of the reference drugs.

The significant reductions in the packed cell volume (PCV) of mice transfected with the malarial parasites may be due to combined effect of plasmodial infection and possible destruction or clearance of plasmodial infected blood cells by the administered extracts (Thomas *et al.,* 1998). The drop in the PCV that is responsible for malarial anemia occurs both through an increase in the rate at which old red blood cells are broken and a decrease in the rate at which new ones are produced. Plasmodium not only causes the rupture of parasitized red blood cells, but stimulates the activity of macrophages in the spleen, which then destroys both parasitized and unparasitized red blood cells (Maimuna *et al.,* 2013).That the malaria-induced reduction in the PCV was completely reversed in mice pretreated with higher dosage (400 mg/kg b.wt) of both ADME and ADFE in a comparable manner to the reference drugs supports the anti-malarial

efficacy of the intervention, thereby lending credence to the folkloric uses of the plant in this regard.

The liver is the major organ used for the detoxification of xenobiotics in the body and as such is subjected to many substances causing oxidative stress. Saponin, flavonoids and tannins have been suggested to act as primary antioxidant or free radicals scavengers that can counteract the oxidative damage induced by malaria parasite (David *et al.,* 2004; Okokon, *et al.,* 2008).

In the present study, there were no significant changes in the GSH concentrations between the extract administered groups (*P*>0.05).The significantly (P<0.001) increased membrane peroxidation, typified by high MDA concentration in parasite-infected, untreated mice might suggest the generation of free radicals in malarial-infected mice (Sandro *et al.,* 2012; Momoh, 2014).In the present study, a strong correlation exist between the blood parasite count and membrane peroxidation such that all the drug/extract treated groups, which recorded lower parasite counts, have significantly reduced malondialdehyde level when compared with the transfected, untreated positive control. That all the treated groups exhibited significant (P< 0.001) reduction in membrane peroxidation could be suggesting the prophylactic effects of the intervention. Both reference drugs produced significant reduction in MDA levels with chloroquine exhibiting the greatest effect. The least significant reduction was observed in mice pretreated with 400mg/kg b.wt ADME while the highest effect among the extracts was observed in animals pretreated with 400mg/kg b.wt. ADFE group. Anemia (a decrease in PCV values) which was observed in the ADME/ADFE-treated mice, despite the lower membrane peroxidation coupled with the observation that GSH concentrations were not affected, could suggest the destruction of plasmodium infected red blood cells by the extracts (Maimuna *et al.,* 2013).

The mechanism of anti plasmodial action of this extract has not been elucidated, however, anti plasmodial effects of natural plant products have been attributed to some of their active phytochemical components (Ayoola *et al.,* 2008; Sofowora, 1980).). Some of these phytochemicals like flavonoids, steroids, alkaloids (detected in *A. digitata*) were reported to have antiplasmodial activity (De Caluwe *et al.,* 2010; Sidibe and Williams, 2002). Earlier studies revealed the roles of reactive oxidant specie in curing malaria by creating an unfavourable environment for plasmodial growth (Borris and Schaeffer 1992; Levander and Ager, 1993) but with *A. digitata,* having reported to be a rich source of antioxidant phytochemiclas (Blomhoff *et al.,* 2010; Brady, 2011), different mechanism might be involved.

5.2   Conclusion

The results obtained from this study reveal that the methanol and flavonoid-rich extracts of *A. digitata stem* bark exhibit antimalarial effect by producing significantly low parasitemia and improved chemosuppression in *P. berghei*-transfected mice. The extracts further produced a positive effect on oxidative stress indices in the malaria-infected rats. It can therefore be concluded that the methanol and flavonoid-rich extracts of *A. digitata* possess good potentials as antimalarial agents thereby lending credence to the ethnomedicinal use of the plant in the treatment of malaria.

5.3 Recommendation

In view of these findings, efforts should be made to further:

i.   characterize the active components of this plant; and

ii. elucidate the mechanisms of action of its components on malaria parasite.

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