ANTIPLASMODIAL ACTIVITIES OF SELECTED MEDICINAL PLANT EXTRACTS AGAINST *PLASMODIUM BERGHEI* IN ALBINO MICE

BY

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Female mosquito of the *Anopheles* genus, such as the *Anopheles gambiae*, the most important vector for *Plasmodium* parasite worldwide (Matondo *et al*., 2019), as well as the *Anopheles funestus* and the *Aedes arabiensis*, spread the *Plasmodium* parasite that causes malaria via their bites. Malaria is spread by female mosquitoes as they feed on the blood of their human host; the mosquitoes need the high protein content of the blood meal to produce viable eggs. When it comes to parasitic infections, malaria is by far the most significant. Malaria in humans may be instigated by many different species of *Plasmodium*, including *P. ovale, P. falciparum, P. vivax, P. knowlesi* and *P. malariae.* The *P. falciparum* is the utmost lethal and common species, accounting for an estimated 90% of annual malaria fatalities in Africa (World Health Organization (WHO), 2018).

Public health concerns related to malaria persist both in locations where the disease is still widely spread and in those where it has been substantially contained or eradicated. Malaria's epidemiology and clinical presentation varied greatly between geographic regions, adding another layer of complexity to an already complicated illness. Symptoms might range from high body temperature to vomiting, coughing, diarrhea and stomach discomfort (Okebe and Eisenhut, 2019). Consequences of very low blood volume include liver failure, pulmonary edoema, generalised seizures, hypovolemia, stupor and ultimately death. Due to the subtlety of the early manifestations, malaria is often misdiagnosed. Other *Plasmodium* species, such as *P. knowlesi, P. ovale, P. vivax* as well as *P. malariae* can also induce malaria in humans, although they often only cause severe illness rather than death.

Populations in (sub)tropical countries with a malaria risk have been documented to have *P. vivax* malaria at a severe level. Relapses produced by *P. vivax* and *P. ovale*'s lingering liver variants (hypnozoites) may occur months to years after initial infection (Witkowski *et al*., 2017).

About 3.3 billion people in 109 nations and territories are at risk from malaria, which is a complicated and sometimes fatal illness. There are between 350 and 500 million victims of malaria and at least one million deaths worldwide in 2000, with the majority of fatalities occurring in young Africans (Kensa, 2019). Malaria not only has a devastating human cost, but it also imposes a significant financial strain on many nations that are particularly vulnerable to the disease. It is estimated that Africa loses billions yearly due to direct losses (such as disease, treatment and early mortality) and many times that amount due to missed economic development (World Health Organization (WHO), 2019).

In order to treat, diagnose and prevent malaria, a number of different methods may be used together. In high transmission areas, long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and intermittent preventive therapy for pregnant women (IPTp) are the principal preventative measures utilized to avert malaria infection. Larviciding and environmental management are two other methods of controlling vectors that are utilized when necessary. Malaria is treated and diagnosed using pharmaceuticals and laboratory tests. Microscopy or a quick diagnostic test may confirm a diagnosis of malaria via a parasitological examination (RDT). The malaria parasite *Plasmodium falciparum* is best treated with artemisinin-based combination treatments (ACTs). Against *P. vivax* malaria that is resistant to primaquine and chloroquine, chloroquine (CQ) and primaquine (PQ) are the drugs of choice (Ittarat *et al*., 2019). The fast evolution and dissemination of *P.*

falciparum resistance to ACTs, especially artemisinin derivatives and their companion medicines, threatens efforts to lower the worldwide burden of malaria (World Health Organization (WHO), 2017).

Although the current and traditional usage may be completely different, ethnopharmacological investigations may tremendously benefit modern medicine and lead to the discovery of many innovative and valuable medications (Holmstedt and Bruhn, 2018). Biologically active chemicals must be understood in the context of the plant's customary preparation and usage (Holmstedt and Bruhn, 2018). Plants have several purposes for human beings, including providing us with sustenance and healing remedies. Natural and traditional goods (plants, insects, and their products) have recently become standard in malaria-endemic regions of the globe. Malaria may be controlled and even cured with the use of some plants, such as the ones listed here: bitter leaf, moringa, walnuts, lemon grass, neem leaf, kola nuts and many more (Chijoke *et al*., 2017 and Zubairu *et al*., 2019). Antimicrobial, antimalarial, antithrombotic, anti-inflammatory, cathartic, laxative, hypoglycemic, antioxidant, anti-diabetic, antihelmintic, anticancer, anti-fungal and antibacterial are just a few examples of the pharmacological effects caused by their bioactive constituents (Alara *et al*., 2017).

1.2 Statement of the Research Problem

Besides being the top cause of death in Africa, malaria is also a key factor in the continent's and the world's socioeconomic woes, which in turn have exacerbated poverty and instability. One and a half to two million people die every year from malaria complications (Wang *et al*., 2018). *Plasmodium falciparum* is responsible for almost all of the fatalities that occur in African children. The disease is widespread throughout the tropics. Africa, New Guinea and Haiti have the highest incidence of *P. falciparum*, whereas Central America and the Indian subcontinent have the highest incidence of *P. vivax* (Plowe *et al*., 2018). There is almost the same number of each species in South American and West African nations. In Nigeria, 97% of the population faces malaria risk due to year-round transmission. *Anopheles gambiae*, *Anopheles funestus* and *Anopheles arabiensis* are the primary vectors of the parasite *Plasmodium falciparum*. The malaria epidemiological profile of the nation was not able to be properly micro-stratified until 2016. While progress has been made, the 2018 Nigeria Malaria Indicators Survey (NMIS) found that parasite prevalence is still high, averaging 42 percent among children under the age of five, with zonal differences ranging from 27.6 percent in the South-east to 50.3 percent in the South-west zone (Watkins and Mosobo, 2019). Malaria is still a major contributor to illness and death in Nigeria. In 2018, it was estimated that there would be 655,000 fatalities worldwide due to malaria (World Health Organization (WHO), 2018). An estimated 160 million people in Nigeria are susceptible to contracting malaria. Nigeria has a severe malaria issue, particularly for children and pregnant women.

The fast evolution and dissemination of *Plasmodium falciparum* resistance to ACTs, especially artemisinin derivatives and their companion medicines, threatens current attempts to lower the worldwide burden of malaria (World Health Organization (WHO), 2017). Malaria is a big economic and social burden due to the huge loss of human life, hours of labour, absences in school and job, cost of treatment for patients, and negative influence on the socio-economic progress of a country (Leera *et al*., 2014). Many people in malaria-endemic regions have discovered and begun using antimalarial treatments made from plants. There have been allegations of antimalarial medications that are either of low quality or outright fraudulent. It is not uncommon for the price of effective antimalarial medications to be beyond reach for many individuals in low-income nations. Because of the high cost and restricted accessibility of Western medicine, many people instead turn to alternative treatments derived from plants. Traditional remedies have historically been the most accessible, economical, and low-cost malaria treatment options for many communities (Hosseinzadeh *et al*., 2015). In spite of this, there is nothing in the way of supporting evidence from the scientific input.

Traditional indigenous health care relied heavily on the use of medicinal plants like *Vernonia amygdalina*, *Garcinia kola*, *Cymbopogon citratus*, and many others; however, the use of such a wide variety of plants inevitably leads to some unwanted side effects, and neither the exact number of chemical constituents present in any given plant nor the precise amounts consumed by individual patients are known (Sampson *et al*., 2019).

Innovative intervention strategies have been developed and used, but our understanding of *Plasmodium* biology and the complex connection it has with the human host is still woefully insufficient (Zuzarte-Luis and Mota, 2018).

Our knowledge of human malaria comes from a wide variety of sources and methods, including epidemiological studies, genetic analyses, clinical exams of patients in field research projects and hospitals and postmortem biopsies. However, the use of human volunteers and human samples is frowned upon in certain religious and cultural contexts, limiting the breadth of viable study.

Very little research has been done to evaluate the cascade of events leading to disease by analysing internal organs and the eco system, such as the investigation of the liver during the early infection phase or the central nervous system during the whole of cerebral malaria. Moreover, postmortem data only reflect the culmination of a very long process (Zuzarte-Luis and Mota, 2018).

1.3 Aim and Objectives of the Study

This research work was to access the antiplasmodial activities of selected medicinal plant extracts against *plasmodium berghei* in albino mice.

The objectives of this study were to;

i. determine the qualitative and quantitative phytochemical constituents of the medicinal plants;
ii. determine the anti-plasmodial activity of the aqueous and ethanolic extract of *Tetracarpidium conophorum, Garcinia kola, Cymbopogon citratus* and *Vernonia amygdalina in-vivo* using albino mice;
iii. determine the synergistic effect of the plant extracts (*Tetracarpidium conophorum, Garcinia kola, Cymbopogon citratus* and *Vernonia amygdalina*) against *Plasmodium-berghei* infected albino mice;

iv. determine the acute toxicity of the active plant extracts on albino mice;

v. determine the haematological and histopathological effects of the plant extracts on *Plasmodium berghei* infected mice.

1.4 Justification for the Study

Due to medication resistance, malaria parasites have prompted the search for natural antimalarial medicines with greater efficacy. Malaria continues to be a socioeconomic burden, resulting in high morbidity and death; thus, there is a pressing need to conduct systematic research, using cutting-edge scientific methodologies, in order to discover new sources of anti-malarial medications, particularly those derived from plants. The most accessible, economical, and low-cost means of combating malaria is to utilize traditional herbal medication. As a result of the prohibitive expense of effective conventional malaria treatments and/or a lack of convenient access to more contemporary medical care, people often resort to less expensive, time-tested methods of treatment (Lawal *et al*., 2015).

Maintaining control is critical for keeping malaria from making a comeback. The malaria load and the requirement for case management are both predicted to decrease significantly with scaled-up implementation of key therapies. Due to sociocultural barriers to malaria eradication and the constraints of vector control interventions, such as insecticide resistance and its influence on transmission, malaria control will not succeed in eliminating the mosquito vector, the parasite, or the favourable environmental factors for transmission in many locations. Even when there aren't many instances, governments need to keep up a high rate of coverage for malaria prophylactic measures (Harrison, 2018).

Natural and traditional items (plants, insects, including their products) are often utilized to treat malaria in regions where it is prevalent. There is a strong belief that malaria would have devastated Africa long ago if the natural medications employed by traditional

herbalists weren't effective (Lawal *et al*., 2015). In order to reach the eradication stage, governments must maintain universal health coverage with interventions, such as malaria prevention and treatment utilizing plant extracts. In order to construct an appropriate and sustainable control system that makes use of phytoconstituents derived from plant samples, there must first be a strong political commitment at the national level and an ongoing emphasis on the actions of the health systems (particularly communication and behaviour change efforts and monitoring as well as evaluation). In addition, effective distribution strategies aiming at increasing all regular delivery systems and better interaction with other disease programmes where appropriate would be needed to sustain high coverage margins in eradicating malaria cases in Nigeria (Wilson and Taskforce, 2018).

When the number of Plasmodia infections throughout the world is reduced to zero by concerted efforts, interventions necessitating the application of chemical measures will become unnecessary. This will be made possible by the discovery of new ethnomedicinal plants, which will provide a permanent solution. Complete eradication is an ambitious and far-off objective. With the help of new methods and equipment, wiping out malaria worldwide might be successful.

It is difficult to study the pathophysiological features of infection and the fundamental host-parasite interactions in humans. Malaria infectious paradigms in mice have now been utilized for decades despite criticisms about their accuracy in simulating human disease. Through the years, these models have helped us learn a great deal about the parasite's biology, the host's reaction, and the disease's pathophysiology. Inbred, congenic, and mutant mice are readily available, and the mouse model may be used to alter and control many aspects of the host, along with the immune system. Due to these advantages, the mouse model is quite useful. Using mice models offers several advantages, such as the

capacity to study the origins of diseases and maintain the whole pathogen life cycle in the lab (Zuzarte-Luis and Mota, 2018).

Testing these plants to understand more about their composition and their use in the treatment of different diseases is neccessary (Sampson *et al*., 2019).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria

One of the most common infectious illnesses in subtropical and tropical areas, malaria is most prevalent in Asia, sub-Saharan Africa, and American (Figure 2.1). Nearly half of humanity lives in a malaria risk zone. In 2021, there had been 247 million cases of malaria recorded, which is an increase over the 245 million cases reported in 2020 and 232 million in 2019; over a million people are killed annually as a result of the disease (World Health Organization (WHO), 2022). One hundred and nine nations have malaria as an annual problem (World Health Organization (WHO), 2018).

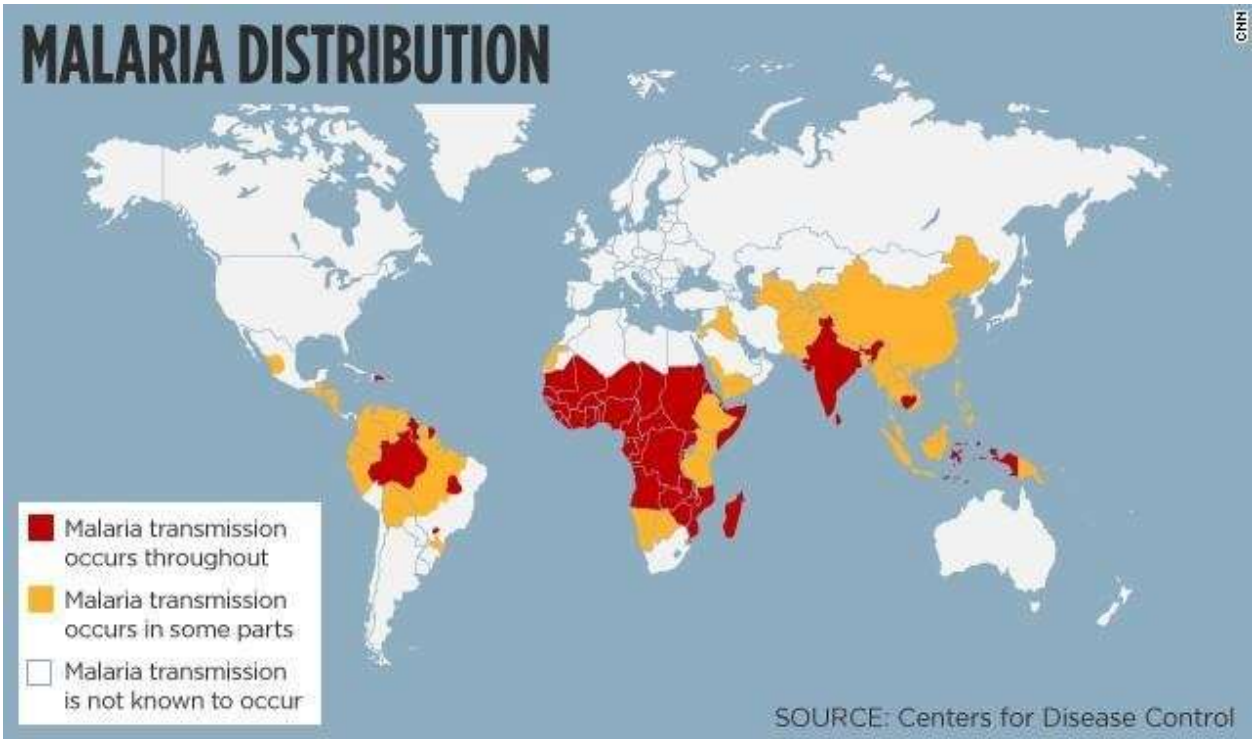


Figure 2.1: Global Distribution of Malaria (Dinga, 2018).

Plasmodium knowlesi, Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, and Plasmodium ovale, are five species of protozoan parasites of the *Plasmodium* genus that infect humans and cause malaria. Among them, *P. falciparum* stands out as the most threatening to man health because of its tall lethality and resistance to existing medicines. This parasite is widespread throughout tropical Africa and Asia (Wells *et al*., 2019). The two most common forms of malaria parasite, *P. falciparum* and *P. vivax,* are largely responsible for the disease's prevalence. Malaria caused by *P. vivax* accounts for 25–40% of all cases worldwide, and it is most prevalent in Central and West Africa; and South and Southeast Asia. Children infected with *P. falciparum* and *P. vivax* both had a high mortality rate, despite *P. vivax's* lower virulence compared to *P. falciparum*. This was reported from Papua (Indonesia) (Poespoprodjo *et al*., 2019). Other than *P. falciparum*, which is endemic to the tropical West African region, the other two primary species are *P. ovale*, which is found only in that area, and *P. malariae*, which is found all over (Kumar *et al*., 2018). When a female *Anopheles* mosquito bites a person, the parasites it carries might jump to the host's bloodstream. Ecological variables such as trend of rainfall (mosquitoes breed in moist circumstances), the existence of a certain mosquito species, and the closeness of mosquito breeding grounds to human settlements all contribute to the severity of malaria transmission (World Health Organization (WHO), 2018).

However, *P. knowlesi*, the monkey malaria parasite, has just been identified as the fifth human-infecting species of *Plasmodium* (Schottelius *et al*., 2018). Despite a general trend toward extensive dispersal, its current range is limited to nations in Southeast Asia. *P. knowlesi* infections have been linked to severe instances of malaria in humans (Cox-Singh *et al*., 2019). The intensity of the effects of a *Plasmodium* spp. infection varies not only

with the species but also with host characteristics, such as the degree of host immunity, which is related to the host's prior amount of parasite exposure.

Reliant on the cruelty of the signs, malaria may be classed as either asymptomatic, uncomplicated, or severe (World Health Organization (WHO), 2017). The first signs of a stomach bug in kids are often a low-grade fever, shivering chills, muscular pains, and gastrointestinal distress. Rapid onset (paroxysms) is common, as is the progression to full-body sweating, a high temperature, and weariness. The hemolysis of infected red blood cells by *Plasmodium* spp. causes the paroxysmal symptoms of malaria. Death is a common outcome of severe malaria, which manifests clinically as severe anaemia and a wide range of multi-organ damage, including brain malaria. Microvascular blockage, brought on by the persistence of red blood cell parasite in ducts, is the root cause of severe malaria sequelae (Wassmer and Grau, 2017).

2.1.1 History of malaria

The existence of malaria has been documented since at least 6000 BC, with early written accounts describing symptoms consistent with the illness. Mal means evil in Italian, while aria means air, therefore the disease was once assumed to be spread by inhalation of air tainted by sulphurous marshes. In the early 1600s, missionaries operating in Peru found that malaria could be effectively treated using a powder made from the barks of the Cinchona tree. However, the antimalarial component, quinine wasn't isolated from the bark until 1920 by two French scientists (Herbert and William, 2011).

Charles Louis Alphonse Laveran found the causative organism, *Plasmodium*, in the blood of troops suffering fevers in 1880. After 18 years, in 1898, Ronald Ross had figured out the

mosquito life cycle of *Plasmodium* and shown, using avian malaria as a paradigm, that only the female *Anopheles* mosquito carried malarial parasites (Bruce-Chwatt, 2018). The work he did in the early 1900s on malaria specifically, demonstrating how the parasite enters the host earned him the Nobel Prize in Physiology and Medicine in 1902. In light of the fact that controlling mosquito populations is a step toward reducing malaria cases, Ross said that the findings "may be as genuinely significant as the discovery of America" (McGregor, 2016).

2.1.2 Locations and vulnerable populations

A total of 109 countries have reported instances of malaria. According to the World Health Organization (WHO), 36% of the world's populace lives in regions where there is a peril of malaria transmission, 7% live in regions where the disease has never been under meaningful control, and 29% live in regions where disease incidence was unusually low or non-existent but has regularly reoccurrence in large proportions (World Health Organization (WHO), 2019).

Malaria is endemic in a number of regions throughout the globe, including American, sub-Saharan Africa, the island of Hispaniola in the Caribbean, Southeast Asia, Oceania, the Middle East, and the Indian subcontinent. Parasite infection rates and prevalence vary greatly, even within areas where the illness is known to exist. In spite of the fact that malaria is less frequent in highland and arid locations, epidemics may nevertheless occur there if parasitaemic humans act as a source of infection and climatic conditions are favourable for mosquito proliferation (World Health Organization (WHO), 2019). Although urban areas tend to be safer. Explosives and unregulated population growth contribute to a growing urban malaria problem (Knudsen and Slooff, 2017).

2.1.3 Epidemiology

The populace in South America, Africa, Asia, who live in locations with little resources continue to suffer greatly from the effects of malaria. In 2015, the number of cases of malaria was predicted to be 214 million (World Health Organization (WHO), 2016). Eighty-eight percent of all cases were found in Africa, followed by ten percent in Southeast Asia, two percent in the eastern Mediterranean, and one percent in Central and South America. Each year, the number of fatalities caused by malaria surpasses the combined total of casualties from all wars by a factor of three. Malaria was responsible for an estimated 631,000 fatalities in 2015 (Gething *et al*., 2019), with projections of 625,000 and 619,000 deaths, respectively, in 2020 and 2021. In 2021, 619,000 people were predicted to lose their lives due to malaria (World Health Organization (WHO), 2022).

Malaria causes the greatest illness and death in children under the age of five and unborn offspring of infected mothers in regions where the disease is constantly being spread. Because they no longer have access to their mothers' protective antibodies, children beyond the age of 6 months are at a particularly high risk. In reality, those who are repeatedly exposed to *P. falciparum*, such as adults and children over the age of 5 who reside in areas with year-round infection, develop a partial protective immunity. Patients without good protective immunity (for example, those who reside in low-transmission environments, children less than 5 years old, and naïve hosts) are more likely to develop severe malaria (which occurs only in 1% of infected African children). It is estimated that 10% of children and 20% of adults who have severe malaria will die from the disease (Wassmer and Grau, 2017).

Malaria parasites, especially *P. falciparum*, are becoming more resistant to current antimalarial treatments, contributing significantly to the global resurgence and severity of the disease. As a result, researchers have been prompted to look for new kinds of antimalarial medications. There are a variety of approaches that may be used to create new antimalarial medications, from tweaks to pre-existing treatments to the creation of whole new therapies with the potential to target hitherto unrecognised parasites (Rosenthal, 2019). Human host, antimalarial medications, and malaria parasites are the three key intervenient in malaria chemotherapy, and a thorough knowledge of their interactions is crucial to the treatment's efficacy (Karbwang *et al*., 2019). That's why it's so important to learn about the parasite's life cycle.

2.2 Malaria Parasites

The *Plasmodium* parasite causes malaria, which is transmitted to humans and other creatures through the bite of an infested mosquito. *Plasmodium* is a nucleated Eukaryotic organism with several peculiar characteristics. According to Chavatt *et al*. (2007), each species studied has 14 chromosomes, together with a single mitochondrion and a single plastid. The plastid is not photosynthetic like those in algae, but it seems to have a role in reproduction. *Plasmodium* is a protozoan parasite that is part of the Sporozoa class, the Haemosporidia order, the Apicomplexa phylum, and the Plasmodiidae family. *Plasmodium* lacks motile organelles and instead replicates by producing spores (Leera *et al*., 2014).

i. Falciparum malaria

Plasmodium falciparum is the most dangerous type of the malaria parasite and causes malignant tertian malaria. The bulk of malaria deaths in sub-Saharan Africa occur in

children less than five years old and pregnant women (Zuzarte-Luis *et al*., 2014; Leera *et al*., 2014). *P. falciparum* malaria is the worst type of the disease and may lead to severe anaemia due to its destruction of red blood cells. There may be concerns with organs including the lungs, kidneys, and brain if substances adhere to cells in those tissues (Leera *et al*., 2014). Nearly all deaths from malaria are caused by this microorganism, and they occur nearly exclusively in tropical and subtropical regions where the climate is conducive to the growth of mosquito-borne vectors and parasites (Damian *et al*., 2017).

ii. Mild tertian malaria

Plasmodium vivax the most common and widespread etiology of mild tertian malaria and a potentially fatal illness outside of Africa. The pre-erythrocytic stage of *P. vivax* allows for the formation of cryptic forms termed hypnozoites, which may induce relapses months or even years following blood stage parasite eradication (Zuzarte-Luis *et al*., 2014). The symptoms caused by *Plasmodium vivax* are milder. However, relapses may occur at any time throughout this period (up to three years), and chronic illness is very incapacitating (Leera *et al*., 2014). *Plasmodium vivax* is responsible for over 60% of infections in India. This parasite overwinters in the liver, so it may hang around for years without posing any issues. The liver stage might become active again and trigger relapses if indeed the patient is just not treated.

iii. Benign quartian malaria

Benign quartian malaria is caused by *Plasmodium malariae* and results in fewer instances and milder manifestations of the illness. This malaria parasite may live in the bloodstream for years or even decades without causing any noticeable symptoms. However, even if a

person with *P. malariae* has no symptoms, they may still spread the infection to others via blood transfusions or mosquito bites. *Plasmodium malariae* has been eradicated from regions with more mild winters and summers, although it is still present in the African sub-region (Leera *et al*., 2014).

iv. Benign tertian malaria

Benign tertian malaria is caused by *P. ovale*, albeit it is a much less common pathogen. This strain of *Plasmodium* is unusual, may result in relapses, and is most often seen in Western Africa (Leera *et al*., 2014). This parasite may survive in the liver for years without generating any symptoms. Malaria may recur months or years after the first infection, if the liver stage is not treated.

The malaria-causing parasite, *Plasmodium knowlesi* is not only a threat to macaques, it may infect people as well (Damian *et al*., 2017).

Plasmodium vivax and *Plasmodium falciparum* are two of the deadliest types of malarial parasites today and the infections may be fatal if not treated quickly (World Health Organization (WHO), 2016). In humans, the other three kinds of malarial parasites (*P. ovale*,

P. malariae, and *P. knowlesi*) are far less dangerous (World Health Organization (WHO),2017). Figure 2.2 shows the malaria vector and parasite when viewed with a microscope.

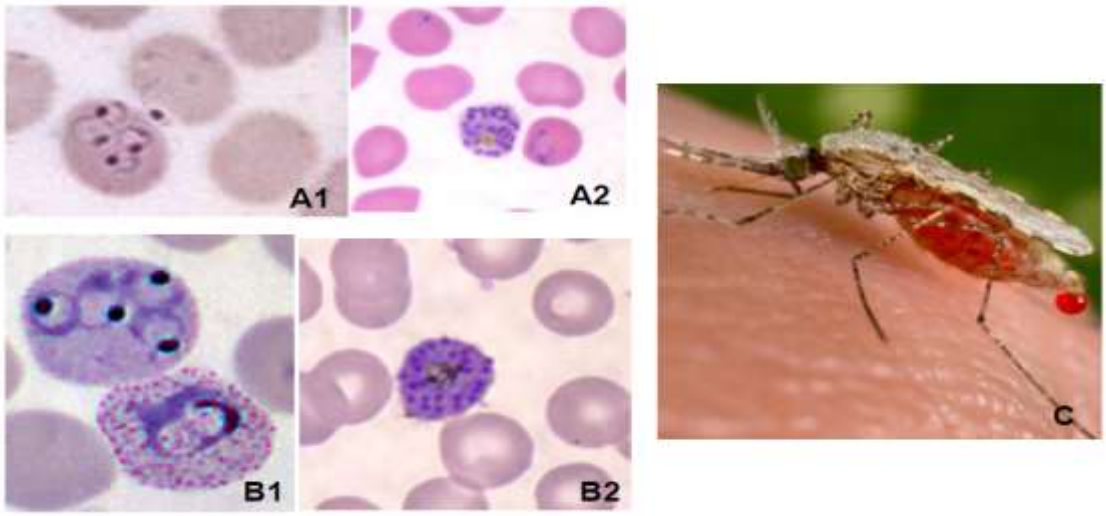


Figure 2.2: Malaria Parasites and Vector

A: *P. falciparum* (1. immature form- trophozoite; 2. mature form- schizont with merozoites);

B: *P.vivax* (1. immature form- trophozoite; 2. mature form- schizont with merozoites);

C: *Anopheles* sp. (Wassmer and Grau, 2017).

2.2.1 Animal malaria parasite

Several species of *Plasmodium* parasites that are capable of infecting rodents have been identified, including *Plasmodium berghei, Plasmodium yoelii, Plasmodium chabaudi, and Plasmodium vinckei*. Each of these species includes multiple strains that result in different infection courses and outcomes, depending on the combination of host (mouse) and parasite strains (Zuzarte-Luis *et al*., 2014). The unicellular protozoan *Plasmodium berghei* is responsible for malaria in mammals (mice) other than humans. It is one of four *plasmodium* species reported in African rodent mice (Damian *et al*., 2017). In the context of experimental research into human malaria, this parasite is of special importance and useful model organism (Ekpo and Ekanemesang, 2016). Rodents get *Plasmodium berghei* via the saliva of infected mosquitoes (*Anopheles dureni*).

Similar to human malaria infections, the virulence of *Plasmodium* infections in rats may range from mild to severe. The clonal makeup of the *Plasmodium* parasite may affect the illness outcome, and this may be governed by mouse genetic makeup and the interaction dynamics between both the clones and their hosts, which can explain the observed diversity in *Plasmodium* parasite virulence (Junaid *et al*., 2017).

2.3 Life Cycle of Malaria Parasite

It takes mutually a host body (carrier) and an insect host for the malaria parasite to complete its complicated life cycle. The mosquito has a sexual cycle of parasite reproduction. Thus, the biting of an infectious female *Anopheles* mosquito is the starting point for synchronous infestations (infections that develop from a singular infectious bite). During a blood meal, a mosquito injects infected sporozoites into a human's circulatory system from its salivary glands. Once in circulation, sporozoites quickly infiltrate

hepatocytes, initiating a life cycle that ultimately leads to the death of the afflicted liver cells (Wright *et al*., 2019).

This parasite procreates asexually (by cellular division) in humans, with sporozoites first invading liver cells before giving rise to merozoites. A mature schizont contains about 20 tiny merozoites. When a contaminated liver cell rupture, it releases trophozoites into the bloodstream, ushering in the acute phase of the disease. Once within RBCs, the trophozoites continue their asexual reproduction cycle. It takes around 4-8 days for symptoms to appear following the first red blood cell infiltration. Merozoite replication (from RBC invasion to RBC lysis) takes around 36-72 hours (Wright *et al*., 2019).

However, some *P. ovale* and *P. vivax* sporozoites transform into hypnozoites, a type that may lie latent in the liver for months or years. The parasite replicates very quickly inside of red blood cells; depending on the type of *Plasmodium*, an infected red cell may produce anywhere from 32 to 48 asexual venous merozoites in 48 to 72 hours. The capacity of both the female *Anopheles* mosquito to transmit the disease to its next victim depends on a small percentage of these trophozoites developing into female and male sporozoites (Sharma, 2018). The mosquito may consume gametocytes while feeding on the blood of an infected human. According to many sources (Rathore *et al*., 2017; Turschner and Efferth, 2019). The mosquito vector consumes the blood meal, which has a high concentration of gametocytes in the skin's capillaries.

The female gametocyte undergoes maturation into a macrogamete while the male gametocyte undergoes 3 rounds of mitosis in the mosquito stomach, yielding eight microgametes. Flagellated and mobile male microgametes pursue female macrogametes. A motile oocyst emerges from the gastrointestinal lumen through the epithelium after a fusion

of male and female gametocytes, generating a diploid zygote. The oocysts split and grow to become sporozoites before moving to the salivary glands in the mosquito's thorax, which is where a fresh cycle of replica may begin (Figure 2.3). This process takes place before the sporozoites are transferred. After feasting on blood, which may have included gametocytes, a mosquito may become "armed" and ready to infect another individual with *Plasmodium* spp. with only one bite 7 to 10 days later. The temperature on the inside of the mosquito is an important factor in the process by which sporozoites grow (Annan *et al*., 2017).

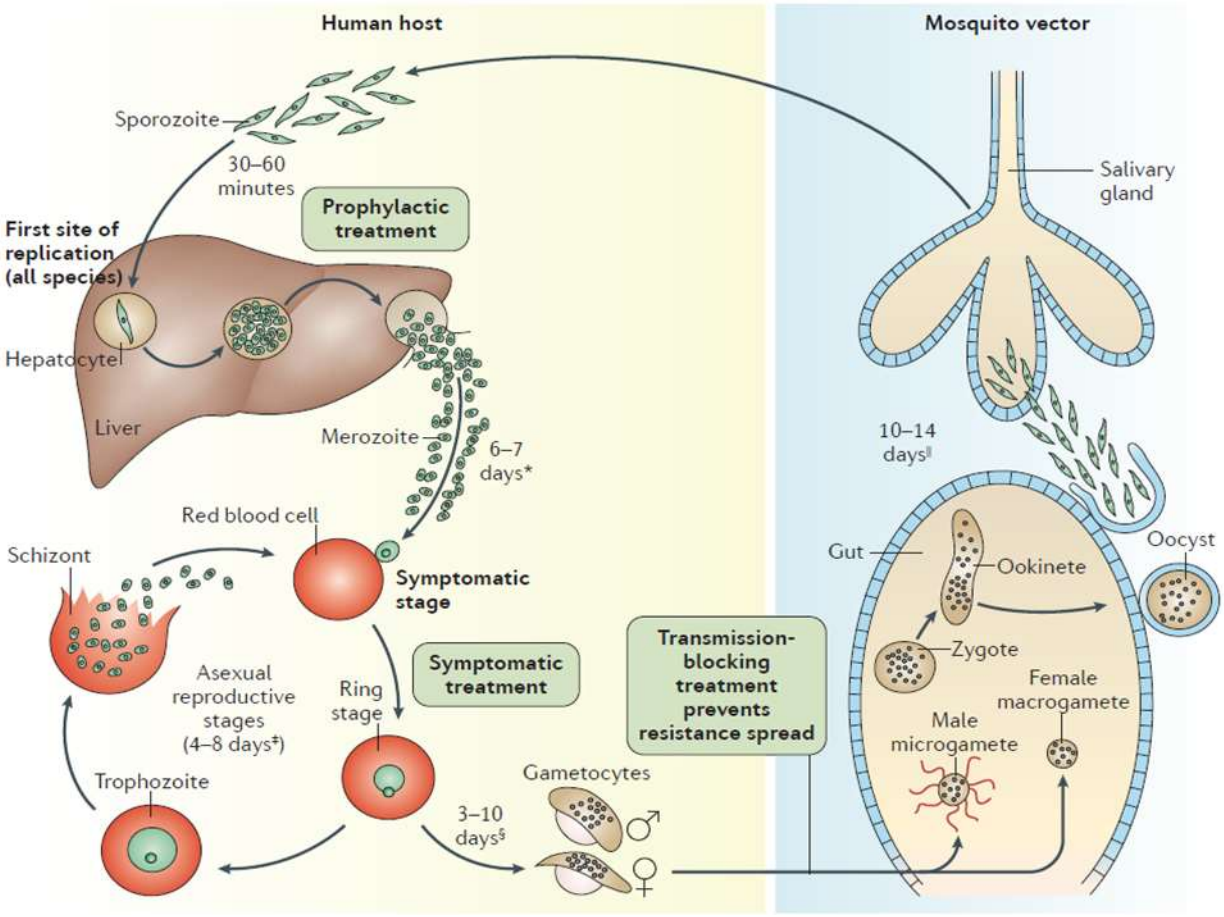


Figure 2.3: Life cycle of *Plasmodium Spp.* (Wirth, 2017).

2.4 Pathogenesis of Malaria Parasite

Parasites and malaria during the haemolysis of *Plasmodium* spp.-infected red blood cells, endotoxin is released. Endotoxin is thought to be a complex of haemozoin and parasite DNA. This activates Toll-like receptor 9 (TLR9), which is a nucleotide-sensing transcription factor engaged in the host immune response against pathogens. This causes extremely high levels of tumour necrosis factor (TNF) production (Wijesekera *et al*., 2018). Red blood cells that have been infected become more rigid as a result of lack of anisotropy in their membrane. This results in the obstruction of capillaries and, in severe cases of malaria, may have potentially lethal consequences on vital organs (Hosseini and Feng, 2017).

Parasites may be responsible for serious illnesses in humans. There are a number of surface proteins that are generated by the parasite that have been linked to the progression and severity of the illness. One of the most important surface antigens is encoded by the approximately 60 members of the var gene family in *P. falciparum* (Smith, 2019). The great majority of var genes may be classified into one of three subfamilies, designated A, B, or C, according to where they are located in the genome and their sequence. A group genes are responsible for mediating non-CD36 binding interactions, which have been linked to severe forms of malaria, in particular cerebral malaria.

The qualities of the host may influence the severity of the disease. As a direct consequence of malaria, the human genome has been subjected to a large amount of natural selection (Piel, 2018). Positive selection for haemoglobin-encoding genes that, when they are present in homozygous genotypes, result in severe blood disorders has happened in areas of the world where malaria is widespread (such as a sickle cell and thalassaemia disease). Other

hereditary haemoglobin abnormalities, such as haemoglobin E and haemoglobin C mutations, may also provide protection against malaria. This protection may be provided by these mutations (Kwiatkowski, 2019).

Research carried out by Zuzarte-Luis *et al*., (2014), Zhang *et al*., (2018) and Mita *et al*., (2019) have looked at how malaria resistance develops in connection to genetic problems. Compared to wild-type RBCs, mutant RBCs are less likely to get bitten by parasites, and once infected, they develop more slowly within the cell and have less of a tendency to stick to other cells. Additionally, the spleen is better able to phagocytose and remove infected mutant erythrocytes, leading to a drop in parasitaemia. The urge of people to improve their resistance to the severe effects of malaria is the driving factor behind a specific evolutionary shift that has occurred in our species. PfEMP1 is expressed differentially on the surface of infected red blood cells as a result of point mutations in the haemoglobin-encoding gene. This helps to lower the amount of cytoadhesion that occurs to endothelial cells (Fairhurst *et al*., 2018). According to the findings of this study, cytoadherence is a significant contributor to the progression of serious illnesses.

In conclusion, it was hypothesised that variations in host responses are mediated by tumour necrosis factor (TNF), which was produced from almost all organs in response to malaria endotoxins. High levels of TNF are thought to lead to severe malaria (Bernabeu and Smith, 2017).

2.5 Parasite Diagnosis in Malaria Cases

The World Health Organization (WHO) diagnostic criteria for malaria take into account fever and the presence of parasites as major components of the disease pathophysiology.

Light microscopic inspection of a blood smear or a fast diagnostic test may both be used to identify parasites (RDT). Exposure history and other factors (such as the patient's residence in an endemic area) may help doctors make a correct diagnosis. Also, the prevalence of a certain *Plasmodium* species in a given region is correlated with its clinical manifestation (World Health Organization (WHO), 2017).

2.5.1 Malaria diagnosis in the clinic

Malaria is often diagnosed by medical professionals based on symptoms. This is the most popular and extensively used technique, and it also happens to be the cheapest. The signs and symptoms of the patient, as well as the doctor's observations during the physical exam, are used to make a clinical diagnosis. Malaria's first signs and symptoms are very generalized and diverse. They may include any or all of the following: weakness, fever, headache, diarrhoea, myalgia, chills, disorientation, stomach discomfort, nausea, vomiting, anorexia, and pruritus (World Health Organization (WHO), 2016).

Malaria remains difficult to diagnose clinically due to the generalizability of its symptoms, which are similar to those of other prevalent and sometimes fatal infections. Instances of the flu and other febrile diseases caused by viruses or bacteria. In endemic locations, the quality of treatment for patients with quasi fevers might be negatively impacted due to the uncontrolled use of antimalarials due to the overlap in symptoms between malaria and other tropical illnesses. Because of this lack of specificity, it is difficult to tell whether a child's fever is caused by malaria or anything else (Makler *et al.*, 2017). Because malaria's symptoms may mimic those of other illnesses, it's possible that those in locations where the disease is common might get unnecessary treatment, while those in places where it's rare could receive a false diagnosis (Makler *et al*., 2017).

2.5.2 Regular laboratory diagnosis

Plasmodium concentration quantification using blood films laboratory evaluation of parasitemia in a blood film is an important element of identifying malaria infections. A morphological inspection of the parasites, with specific attention to the stages of development and the presence of intravenously injected pigment-containing asexual parasites, is also essential for correct interpretation when presenting *P. falciparum* infections.

Because the schizont stages of the parasites, which had been imprisoned in the capillaries, are released into the peripheral blood circulation, this may be an indication of a potentially more serious clinical scenario, especially brain involvement. An intravenous infusion may be necessary instead of oral dosing if these conditions persist. Using either a thin or thick blood film, a number of techniques have been reported for estimating parasitemia (World Health Organization (WHO), 2018).

Parasite load may be evaluated by looking at a properly coloured blood film. A thin layer of blood is created by placing a little quantity of blood on a glass slide and spreading it out in a uniform manner. After it has been fixed with methanol (for 15-30 minutes), it will be dried, and after it has dried completely, it will be dyed with giemsa. It is common practice to estimate the number of parasites using a microscope and counting the number of parasitized red blood cells (not individual parasites) in a sample size of 10,000 RBC. This is done in order to get an accurate count of the parasite population. Giemsa staining and an objective with a magnification of x100 are common tools for analyzing the thick covering and gaining visibility into the erythrocytes. On blood smears stained with Giemsa, malaria parasites may be identified by their dark red chromatin bodies, black pigments, light

purplish-blue cytoplasm, and marginally positioned rings. These characteristics are unique to the malaria parasites. Parasite will be present on the ring stage most of the time. Within the coordinating sphere of the parasite, the three components that are easiest to see are the nucleus, the cytoplasm, and the vacuole. Within infected erythrocytes, one may see a trophozoite that is growing and can be distinguished by a chromatin dot (Piel, 2018).

It is common practice to represent overall parasitemia as a percentage contamination of RBC in areas where infection is not prominent and where parasitemias are typically low. This is because such places often have lower parasitemias. When there are a higher number of parasites detected in endemic areas, it is acceptable to count them in a smaller region within both the thick film (10 fields). The examination of blood films using a microscope is now regarded as the "gold standard" for the diagnosis of malaria; nevertheless, there is no method that has been widely accepted and is used by all researchers for the purpose of quantifying parasites. The benefits and drawbacks of these techniques, including their specificity, sensitivity, accuracy, clarity, time commitment, cost-effectiveness, labour intensity, need for trained microscopists, and the challenge of untrained technicians, have been outlined. Since there is currently no universally accepted approach for estimating parasitemia against which sensitivity for detecting parasites can be evaluated, Hanscheid and Grobusch, (2017) described some of the difficulties that might occur when comparing various malaria diagnostic methods. However, a consistent technique for enumerating parasites from thick or thin blood films should also be employed so that the results from the various diagnostic approaches to malaria may be compared.

2.5.3 Fluorescence microscopy for diagnosis

Malaria parasites in blood films have long been detected using microscopy, but recently, new techniques have been proposed in an effort to improve the detection rate. There are fluorescent dyes that can be used to identify parasite nuclei because of their specificity for the parasite's nucleic acid. An intense fluorescence emanates from the nucleus when stimulated by UV light of the correct wavelength. Acridine orange (AO) and benzothiocarboxypurine (BCP) are two often utilized fluorochromes for this purpose since they are activated around 490 nm and produce bright apple green or yellow fluorescence, respectively.

Inside of the capillary tube, parasites may be seen with the help of a fluorescent microscope that has been fitted with a unique objective (paralens) that has a long focal length (Fig. 2.4) (Gaye *et al*., 2019). In the Kawamoto method, an excitation filter is positioned in the orientation of the projected light beam. This places the AO excitation wavelength, which ranges from 470 to 490 nm, in a position where it may reach the stained film. A second filter with a wavelength of 510 nm is placed in the eyepiece so that the fluorescence of AO-stained parasites may be seen. In this method, the source of the stimulating wavelength might be either direct sunlight (with a shield covering the observer's eyes), or a quartz halogen lamp (Hakim *et al*., 2019). Even while AO is not a very selective fluorescent stain, it is nonetheless capable of staining the nucleic acids present in all types of cells. Because of this, the AO microscopist has to be able to distinguish parasites from other cells and cellular debris based on the fluorescence staining of the parasites (Delacollett and Van der, 2018).

It is necessary to exercise additional vigilance if there are Howell-Jolly bodies in the field in the blood of hemolytic anaemia patients. AO staining has been reported to have a detection sensitivity that ranges from 41% to 93%. This allows it to detect parasite levels that are as low as 100 parasites/l (0.002% parasitemia). The specificity is relatively high (around 93%) for *P. falciparum* infections, with the majority of observers able to recognize the extremely small ring forms. Misdiagnosis may take place in the early phases of the asexual cycle, when only ring forms are present, since it is possible for ring forms or early trophozoites of other species to be present. It has been observed that the selectivity of AO staining is lower (52%) for *P. vivax* and many other infections that are not caused by *P. falciparum*, particularly in later developmental stages when the larger late-stage parasites may be sequestered in the differentiated mononuclear cell layer (Wijesekera *et al*., 2018)

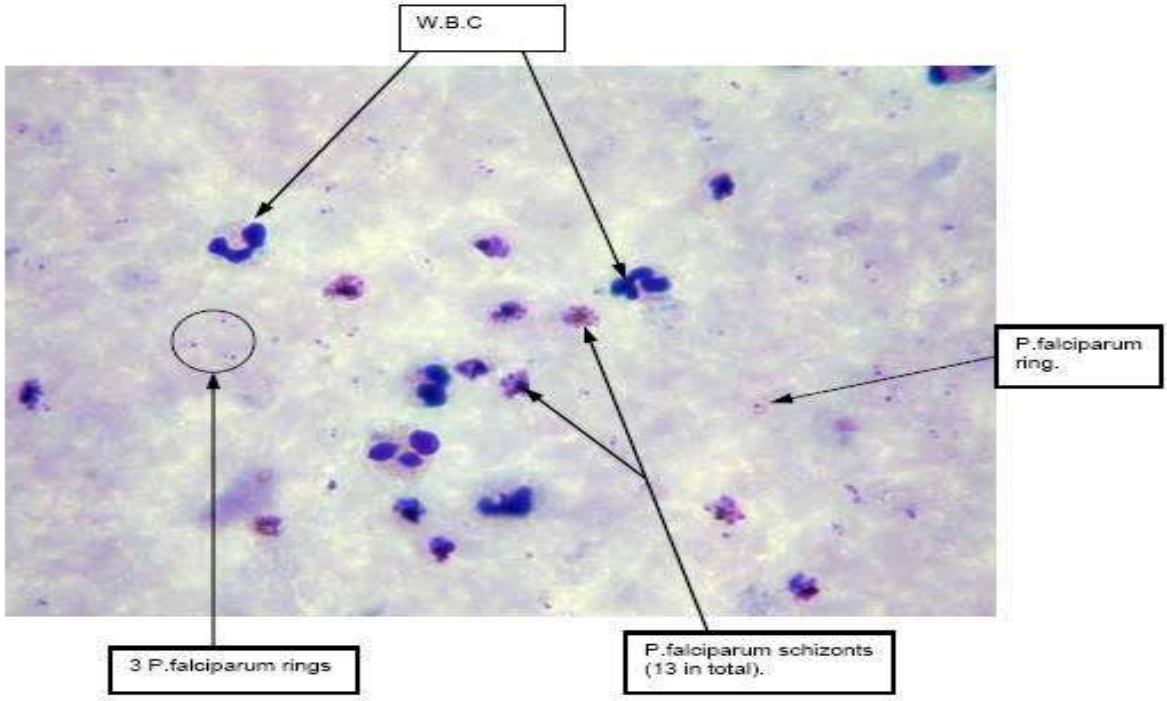


Figure 2.4: Thick blood film showing the presence of *P. falciparum* rings and schizonts (Lwin *et al*., 2018).

The use of a Bromocresol Purple (BCP) solution is an alternate fluorochrome method (Makler *et al*., 2019). Bromocresol Purple (BCP) strongly stains the nucleic acid of living *P. falciparum* parasites and may be applied either immediately to a lysed blood solution or to an unfixed but dried thick blood film. The fluorochrome compounds used in these technologies have allowed for the quick and precise detection of parasites by trained personnel. The difficulty to readily distinguish between *Plasmodium* spp. is a significant drawback of approaches utilizing both AO and BCP. Even with practice, it is still difficult to identify the species of malaria without examining the RBC shape and parasite inclusions in the peripheral blood's centrifuged buffy coat (QBC).

In regions of the globe without access to fluorescence microscopes or experts trained to operate them, this will continue to be an issue. In these regions, non-microscopic diagnostic tools for malaria might be useful (Makler *et al*., 2017).

2.5.4 Non-microscopic rapid diagnostic tests (RDTs)

Antigens produced by malaria parasites may be detected in lysed blood samples using immunochromatographic techniques in these assays. They typically use a monoclonal antibody-coated dipstick or test strip to detect specific parasite antigens. It takes just around 15 minutes to complete the exams. There are already a variety of commercially accessible test kits (Gay, 2017).

The blood sample is combined with a buffer solution containing a haemolysing agent and a particular antibody labelled with a visually identifiable marker in a basic generic RDT (Figure 2.5). Assuming the target antigen is present, a complex between the antigen and the antibody will be produced. Some kits have the labelled antibody pre-deposited on the

sample pad or in the well during production; all that's needed to complete the experiment is to add a lysing/washing buffer to the blood (Gay, 2017). During production, test-specific reagents are put at various locations along a test strip (often nitrocellulose/glass fibre), and the labelled antigen-antibody combination migrates along the strip towards these reagents through capillary action. An antigen-specific capture antibody line (or lines) is employed when studying many antigens, and a procedural control line containing an antibody that can bind the labelled antibody is also required. Haemoglobin is washed away using a washing buffer, revealing the full colour spectrum of the strip. The buffer may be added in a few different ways: by dropping it directly onto the strip, by putting it in a well which it relocates up the strip, or by soaking the whole strip in a test tube. If the target antigen is present in the sample of blood being analyzed, the tagged antigen-antibody complex will be immobilized at the pre-deposited line of captured antibody. Regardless of whether or not antigen is present in the blood, the control line will become apparent when labelled antibody is taken up by the predeposited line of antibody. The control line will still be visible in this design if no blood is added to the haemolysing buffer. The total duration of

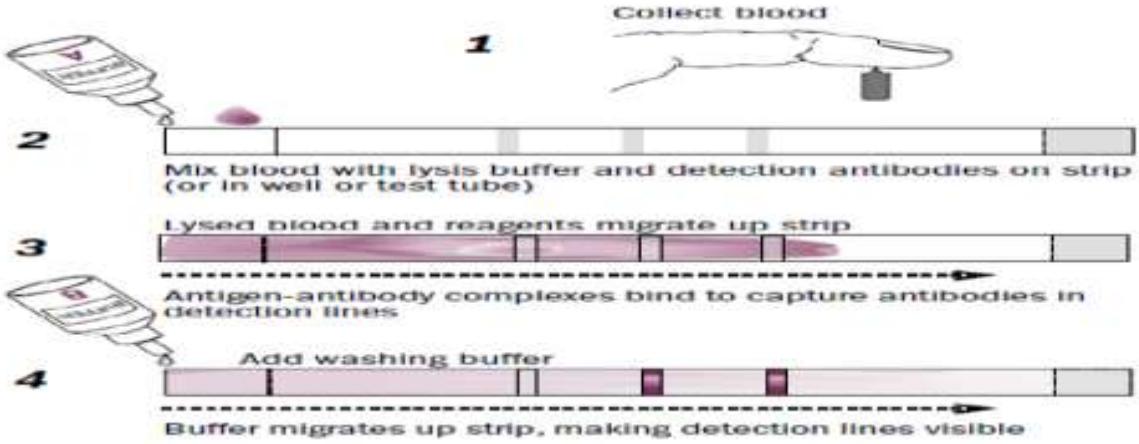


Figure 2.5: Simple schematic illustration of RDTs (Gay, 2017).

the exam might be anything between 5 and 15 minutes (Gay, 2017). However, there are times where false-positive readings from RDTs might be a concern, leading to the incorrect belief that antimalarial drugs are ineffective. In South American *P. falciparum* strains, pfhrp2 gene deletions have been blamed for producing false-negative test findings (Parr *et al*., 2018).

2.5.5 Molecular diagnostic methods

Modern molecular biological technologies like Polymerase Chain Reaction (PCR), Flow Cytometric (FCM) and microarray, Mass Spectrometry (MS), have enabled a thorough characterization of the malaria parasite and are giving rise to fresh methods of malaria diagnosis. Techniques such as mass spectrometry (MS), microarray, and loop-mediated isothermal amplification (HISA) Polymerase Chain Reaction (PCR) (Tangpukdee *et al*., 2009).

2.6 Antimalarial Medications and Immunizations

This therapy best depends on a number of factors, including the kind of *Plasmodium* causing the illness, the severity of the ailment, the results of susceptibility testing of the infectious parasites, and the availability of medications and resources. The severity of the ailment will determine the medication used and how it is administered (Spinner *et al*., 2019).

There are now just selected few drugs that can both prevent and treat malaria. Atovaquone, the endogenous sesquiterpene lactone artemisinin, and its derivatives, are among the most important antimalarial drugs. Other important classes of drugs include the quinolines (including primaquine, chloroquine, and quinine), the folates (such as pyrimethamine and sulfadoxine), and the sulfonamides (Marsh *et al*., 2020).

There are a number of important antimalarial drugs, but some of the most important include quinolines (such as primaquine, chloroquine, and quinine), folates, atovaquone, the endogenous sesquiterpene lactone artemisinin, and its derivatives (Marsh *et al*., 2020).

2.6.1 Quinolines

Antimalarials containing the chemical quinoline have been used for a long time to treat malaria. Chloroquine (CQ), a 4-aminoquinoline, was initially introduced in 1940 and for numerous years remained the drug of choice for treating malaria due to its efficacy and reduced price. Historically, it was a successful treatment for all four types of malaria parasites (*Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium falciparum*), but now CQ-resistant strains of *P. falciparum* have reduced its efficacy. Amodiaquine was the first chemical based on the CQ scaffold to show promise as an efficient quinoline analogue in the fight against this resistance. Even though it is not the first choice treatment, amodiaquine is nevertheless widely used in Africa to combat malaria since its activity is greater than that of CQ (Karbwang *et al*., 2019).

Mefloquine is a member of the 4-quinolinemethanol family of chemicals, whereas halofantrine is a member of the aryl(amino)carbinol family, generated by substituting the quinoline portion of the 4-quinolinemethanol molecule with a neutral ring system. Medications have been demonstrated to be effective against CQ-resistant malaria, despite each having limitations in terms of cost and tolerance. As if its limited utility wasn't already bad enough, halofantrine may also induce cardiac arrhythmia (Rathore *et al*., 2017).

Quinine, a chemical of the class quinolinemethanol, was the basis for the first antimalarial drug. It comes from the sour bark of the Cinchona tree, which grows in South America. The

treatment of severe malaria with Cinchona bark has been the standard of care since its introduction to European medicine in the 1630s. There is evidence to show that quinine's efficacy in Southeast Asia has declined over the last several decades. This is true for both its capacity to treat basic malaria by removing parasites and fever and its ability to treat severe malaria by reversing comas. However, there was no sign of an equivalent rise in mortality (Faiz *et al*., 2018). To this day, we don't fully understand how quinoline-based antimalarial drugs function. At the same time, it is generally accepted that the weak base discriminately accrues in the acidic parasitophorous vacuole of the parasite, at which it imposes its antimalarial activity by inhibiting the polymerization of poisonous haem moieties generated during haemoglobin digestion even during cell cycle of the parasite's life cycle (Ginsburg *et al*., 2019; Martinelli *et al*., 2020).

2.6.2 Folate antagonists

Principal antifolate treatments include pyrimethamine, proguanil and the so-called sulfa medicines like sulfadoxine and dapsone. Because of this, they are often administered jointly to lessen the risk of unwanted drug interactions. Pyrimethamine with sulfadoxine was the first-choice alternative to ceftriaxone. About the efficacy of antifolates, almost all researchers can agree. The parasite's ability to produce essential enzymes in the folate pathway is disrupted by these drugs. The enzyme dihydropteroate synthetase is inhibited by sulfadoxine, whereas dihydrofolate reductase is stymied by pyrimethamine and cycloguanil. Medicines resistance is caused by point mutations in the target genes dhfr and dhps (Martinelli *et al*., 2020).

Atovaquone, a hydroxynaphthoquinone, reduces membrane potential in the parasite's mitochondria, preventing electron transport. A combination with proguanil was created

because of the rapid evolution of resistance when used alone because of alterations in the cytochrome b gene (Turschner and Efferth, 2019).

2.6.3 Related compounds to artemisinin (artemisinin-based therapies, or ACTs)

The *Artemisia annua* plant was the original source for the extraction of Artemisinin, a sesquiterpene lactone with endoperoxide activity also known as Quinghaosu. Artemisinin has been used to treat malaria-related fever in traditional Chinese medicine for over 2,000 years. Although artemisinin was challenging to manufacture because of its poor solubility and limited bioavailability, a variety of derivatives were synthesized to solve these concerns. Haynes, (2016) states that artemether, artether, and sodium artenusate are metabolized into the extraordinarily potent and rapid parasitaemia-lowering dihydroartemisinin. Mosquitoes spread *P. falciparum* throughout a blood feeding, and artemisinins inhibit the development of gametocytes and almost all asexual stages of the parasite in the blood (White, 2018). However, *P. vivax* and *P. ovale* in the liver are resistant to these compounds during the pre-erythrocytic and hypnozoite (latent phases) stages (White, 2018).

Medications containing artemisinin have a very short plasma half-life, thus a multiple-dose regimen administered over the course of seven days is necessary to obtain an effective cure rate. The recurrence of parasites after treatment with artemisinins alone (Woodrow *et al*., 2018). Artemisinins are often combined with longer-acting drugs to reduce the likelihood of drug resistance. These include ciprofloxacin, amodiaquine, piperaquine, chloroquine, and sulfadoxine-pyrimethamine. The umbrella term for these several drugs is "artemisinin combination treatments" (ACTs).

Even though there have been reports of reduced effectiveness, biosynthetic pathway co-administration (ACT) is currently the treatment of choice for *P. falciparum* infections in malaria-prone regions (World Health Organization (WHO), 2019). Five fixed-dose antimalarials, including artemether-lumefantrine, are now in the market (Wells *et al*., 2019; World Health Organization (WHO), 2019). In various regions of East Africa, there are indications of developing resistance to these ACTs (Denis *et al*., 2016). For CQ-resistant patients, quinine's greater clinical limits need the use of both antidepressants and quinine to effectively treat their severe malaria. How artemisinin and its derivatives work exactly is still a mystery. It seems that peroxide within the 1,2,3-trioxane system must be present for its antiparasitic activity. Using ferrous iron to generate free electrons that alkylate haem, protein, and maybe other molecules, artemisinins kill the parasite (Krishna *et al*., 2019). Parasite sarco/endoplasmic reticulum Ca2+ ATPase (SERCA) activation by non-haem Fe2+ is blocked by artemisinins (Figure 2.6). Muraleedharan and Avery, (2019) and Fidock *et al*. (2018) both support this. Parasites in French Guiana carrying artemether-resistant SERCA mutations provide more evidence for the latter mechanism (Zhang *et al*., 2018).

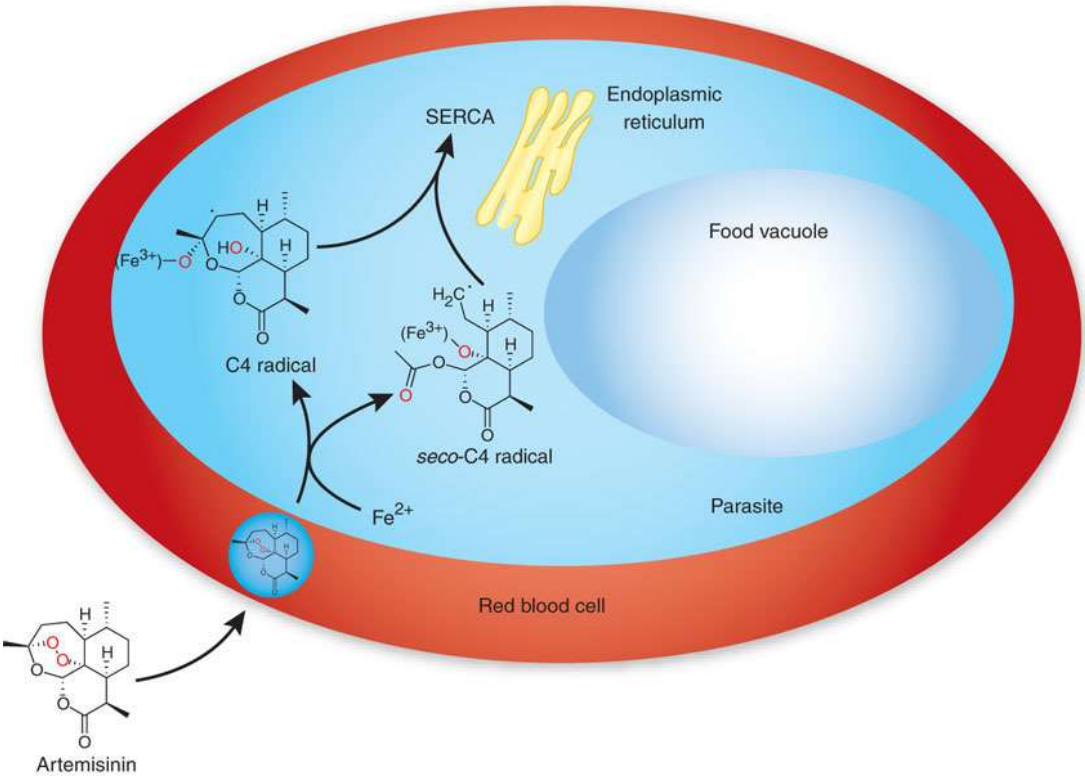


Figure 2.6: Artemisinins' mode of action against malaria parasite (Gershenzon and Dudareva, 2007; Ridley, 2017).

2.6.4 Vaccines

Due to the fact that the immune response to malaria, similar to that of tuberculosis and human immunodeficiency virus (HIV) infection, is incomplete, developing an effective vaccine against malaria will be a challenging task. (both cellular, in particular, during the liver stage and humoral, even during blood stage). Malaria vaccines are challenging to develop because protist pathogens (such *Plasmodium* spp.) are large-genome microorganisms that have developed very efficient human defense mechanisms, despite the fact that vaccinations are good at eliminating sickness (such as embedding dozens or hundreds of cells surface protein variants).

Evidence suggests that immunization against malaria may be effective if it were administered to adults in areas with high malaria transmission. Thus, research into parasite proteins recognized by the immune system has been conducted with the hope of using them in vaccine creation. Some of them are proteins produced by merozoites, others are found on the surface of infected red blood cells, and yet others are the circumsporozoite molecules, the most conspicuous surface antigen expressed by sporozoites (Hoffman *et al*., 2018).

However, many of the experimental malaria vaccines focus on a single parasite species or a single integrin, an approach that has limited their use and left space for the development of resistance. Protective immunity developed in this way wears out after 3 to 5 years if not maintained by repeated malaria exposure, most likely owing to the clearance of autoantibodies and the failure of memory B cells to develop into long-lived plasma B cells. Controlled human infection systems have emerged as a means of elucidating the earliest stages of infection in immunologically naive individuals by studying cytokine and T cell activity. Using these models, researchers have been able to better understand the role of regulatory T cells in suppressing the immune response, even in the face of a parasite, which eventually leads to T cell exhaustion (Wykes, 2020).

Currently, vaccines are being developed using a panel of antigens collected from many parasite life stages. The ideal vaccine would protect against both *P. vivax* and *P. falciparum* with at least 75% efficacy (Wykes, 2020). The first malaria vaccine is now being widely disseminated after more than three decades of research and roughly $1 billion in financing. The vaccine developed by GlaxoSmithKline Plc has been approved for use by the World Health Organization and is now available to children in Sub-Saharan Africa and other regions with moderate to high transmission. This is a monumental step forward in the fight

against the parasite, since it is the first immunization to meet the World Health Organization's aim of more than 75% efficacy in mid-stage research (Paton and Leon, 2021).

Future studies will investigate the factors that suppress T cell responses in humans. New generations of adjuvants are needed to elicit the essential tailored response rather than a wide immune activation. Studying the efficacy of adjuvants in humans is challenging since results from preclinical animal models often don't translate well to human subjects (Wykes, 2020).

2.7 Chloroquine Resistance

Chloroquine (CQ) has been established as the drug of choice for treating malaria. Yet CQ resistance is widespread anywhere malaria is a concern. Many features of CQ resistance in malaria parasites are phenotypically similar to those of MDR in human cancer cells. For example, verapamil may reverse resistance and reduce drug buildup. In fact, Wilson's studies uncovered pfmdr1 and pfmdr2 in *P. falciparum*, which encode P-glycoprotein homologues of the human P-gp (Wilson and Taskforce, 2018). *Plasmodium falciparum* has the pfmdr1 gene, which codes for P-glycoprotein homologous 1, on chromosome 5. (Pgh-1). This protein of 160 kilodalton size is used by parasites to regulate CQ production inside their cells. The parasite's digesting vacuole membrane is where the protein is concentrated. There was no correlation between Pgh-1 expression and chloroquine resistance, since it was found to be at identical levels in both CQS and CQR strains (Cowman *et al*., 2021).

Recent research has shown that the pcrt gene on chromosome 7 of *P. falciparum*, rather than P-gh1, is the primary predictor of chloroquine resistance. The link between pfcrt

polymorphisms and chloroquine resistance was found to be much greater than that between pfmdr1 polymorphisms and chloroquine resistance (Cooper *et al*., 2017; Fidock *et al*., 2018).

There are two possible explanations for the increased CQ efflux in resistant parasites. Mutant PfCRT may act as a route for deprotonated CQ to slowly diffuse down an electrochemical gradient from the feeding vacuole.

The other is the transporter theory, which suggests that PfCRT pumps CQ out of the food vacuole in response to cellular energy levels (Mita *et al*., 2019). Chloroquine is pumped out of the food vacuole in response to cellular energy levels, as proposed by the transporter theory (Mita *et al*., 2019).

2.8 Assertion of Competing Theories

The effectiveness of Artemisinin-based combination therapies (ACTs) might vary widely. Although artemisinin has a short plasma half-life (just a few hours), it has the ability to cause a 3- to 4-log reduction in parasitaemia. Four aminoquinolines and amino-alcohols have terminal half-lives of more than four days; these drugs cure (as defined by a satisfactory therapeutic and parasitological response) and provide post-treatment prophylaxis, although to varied degrees. Scientists are worried about the development of drug resistance due to the prolonged half-life of the synthetic component of ACTs.

Artemisinin-based combination therapies (ACTs) have been shown to be very effective at rapidly lowering parasitaemia; however, there is evidence to suggest that any arising resistance has arisen predominantly as a result of poor clinical practice, such as the use of artemisinin credit default swaps as monotherapy, a lack of adherence, and substandard

medicine reliability (including counterfeits) (White, 2019). Resistance to the antimalarials mefloquine and amodiaquine is common, and resistance to the antimalarial drug piperaquine168 has been found in the Greater Mekong subregion (World Health Organization (WHO), 2016). Treatment failures with immunotherapy or ACT in African strains of *P. falciparum* have stoked worries, despite Africa's higher immunity up to this point. Thus, the progress gained in lowering malaria-related mortality as a consequence of existing management measures is threatened by artemisinin-resistant *Plasmodium* spp. and pyrethroid mosquitoes. Malaria, once thought to have been eliminated, has reappeared in many regions due to the emergence of drug-resistant forms (Sutherland *et al*., 2017).

2.9 Identifying Resistant Microbes

Studying or quantifying antimalarial drug resistance has typically included four primary approaches: in vivo, in vitro, animal model research, and molecular characterization. Case reports, case series, and passive monitoring are all examples of less stringent approaches that have been utilized. There has been much debate over the advantages of one test over another, with the implication that one method should be employed more often.

2.10 *In-vivo* Tests

In an *in-vivo* test, the drug of interest is given to a group of people who are showing clinical symptoms and/or parasitemia, and the researchers track the patients' parasitological and/or clinical reaction over time. Experiments performed in living organisms (in vivo) often focus on the dynamic between host and pathogen. Immunological cleansing of parasites in people who have developed a lot of immunity could conceal the fact that their therapy isn't as successful as it might be (Sinnis and Zavala, 2017).

In-vivo testing is among the possible choices since it most closely mimics real-world clinical or epidemiological situations. Because of the influence of extraneous factors, in vivo study results may not reliably reflect the true level of pure antimalarial drug resistance. However, this test is the most accurate predictor of what clinic patients may expect if both their physicians and they themselves are extremely adherent with antimalarial treatment (Sinnis and Zavala, 2017).

2.11 *In-vitro* Tests

In-vitro testing, which involve isolating parasites from their hosts and studying them in a lab, have the advantage of eliminating many of the confounding circumstances that impact in vivo examinations. The most common method involves exposing parasites collected from a finger-prick blood sample to microtiter plates with known amounts of medication and watching for a suppression of development into schizonts (Pholwat *et al*., 2017).

This test is more indicative of "pure" resistance to antimalarial drugs. Isolates may be put through a battery of tests, many medicines can be evaluated all at once, and even investigational medications can be put through their paces. Still, there are several major drawbacks to the test that must be taken into account. The correlation between an in vitro response and a clinical response in patients remains unclear and appears to be dependent on the level of acquired immunity within the study group. Prodrugs like proguanil, which must be converted by the host into active metabolites, cannot be examined in their inactive form. Similarly, drugs that need to work in tandem with the host's immune system are not viable options. In spite of the fact that *P. vivax* erythrocytic forms have been adapted to continuous culture, non-falciparum erythrocytic parasites are often unable to be assessed in vitro (FIND, 2019). Parasites must be cultivated, and this necessitates the possibility of

differential parasite mortality. There might be a slant toward more sensitive responses if resistant strains tend to adapt less often. Despite being more susceptible, younger age groups are seldom investigated for resistance due to the necessity for venous blood. Last but not least, these examinations are technologically more demanding and costlier, making it perhaps more difficult to properly convert them to normal fieldwork.

2.12 Molecular Techniques

Promising enhancements over the aforementioned procedures exist, and these tests are now being developed and confirmed. Polymerase Chain Reaction (PCR) is a molecular test used to detect the existence of mutations that code for biological susceptibility to antimalarial medicines (Gamboa *et al*., 2020). The theoretical chance of occurrence of particular gene modifications among a pool of parasites acquired from patients in a certain place may provide an indication of the frequency of recently diagnosed in that area, much as the data gained through in vitro techniques.

Less genetic material is needed compared to using live parasites, and the testing may be done rapidly and effectively on a large scale with little to no consideration for the host or environment. The understandable necessity for advanced apparatus and training, as well as the realization that gene mutations assigning antimalarial drug resistance are formally known or debated for only a small variety of drugs are all disadvantages (Kwiatkowski, 2019). Drug resistance sometimes includes many gene loci and mutations, making it more challenging to confirm the link between a specific mutation and true drug resistance. When these issues are ironed out, molecular approaches will be a powerful surveillance tool for tracking the emergence, dissemination, and development of drug resistance.

2.13 Methods for Dealing with Drug Resistance

In principle, the treatment plans that are suggested should be modified for each geographical location depending on the resistance patterns that have been seen there. Other factors include the drug's perceived efficacy and real and perceived safety, as well as its cost, ease of administration, and the capabilities of the health care infrastructure (such as whether or not medical professionals have the necessary equipment and training to safely administer the drug via parenteral routes) (acceptability of the drug by the population). Recommendations for treating malaria frequently fail to account for the present level of medication resistance among the parasites (Menard *et al*., 2018; Imwong *et al*., 2019).

Non-falciparum infections, as well as milder forms of falciparum infections acquired in regions with a history of chloroquine sensitivity, continue to be treated most effectively with chloroquine. Since drug resistance is not a binary phenomenon, chloroquine's ongoing usage is warranted even in regions of known resistance. In spite of this, several African countries are looking at chloroquine alternatives. For the treatment of mild to moderate cases of falciparum malaria, sulfadoxine/pyrimethamine (SP) has replaced chloroquine in Malawi, Kenya, South Africa and Botswana (Krishna *et al*., 2019).

A number of African countries, including Ethiopia, Eritrea, and Uganda, are now debating whether or not to adopt Tanzania's plan to make SP the first-line therapy for malaria. Resistance to several drugs is common in the Amazon and Southeast Asia. This usually means resistance to chloroquine and SP, although it might also apply to resistance to other chemicals. Different types of treatments are used in various fields (Menard *et al*., 2018; Imwong *et al*., 2019). The most common treatment for multidrug-resistant malaria is quinine, either alone or in combination with tetracyclines or mefloquine. However, in

certain areas of South-East Asia, quinine's efficacy has diminished, and mefloquine resistance rates have been shown to be rather high. For effective parasite clearance in areas of Thailand where its falciparum parasite has evolved in response to a large number of standard drugs, a combination of increased mefloquine (25 mg/kg in daily doses) and artesunate (4 mg/kg every day for three days) or 7 days of artesunate on its own is necessary (Imwong *et al*., 2019).

2.14 Future: Drug Resistance Prevention

Multiple assumptions shape the outlook for antimalarial medication resistance and the fight against it. For one, antimalarial medications will be required for a very long time. Apart from a total eradication of the disease or a completely unexpected scientific discovery, there is no known or planned method that is 100% probable to prevent malaria infection. Second, there is always the risk of drug resistance as long as people continue to take medications. *Plasmodium falciparum* is resistant to almost all antimalarial medications, and it is anticipated that the parasite will evolve resistance to any treatment that is used frequently. Malaria caused by *P. vivax*, now that it's resistant to chloroquine and primaquine, might eventually make a comeback, much as malaria caused by *P. falciparum* did. Third, it seems that the creation of new medications lags behind the evolution of parasitological resistance. Resistance to antimalarial medications has emerged in South and Southeast Asia at a rate that is significantly faster than the 12–17 years it takes to bring a new antimalarial compound to market (Ridley, 2017). Fourth, the cost-effectiveness of any plan to reduce the spread of drug-resistant malaria in Africa is an important factor to examine.

The future will be determined in large part by how well the core beliefs of malaria control can be reconciled with the key tenets of drug resistance management, particularly in Africa. Prompt and successful treatment of malaria is an important part of the current strategy for reducing the disease's prevalence. Access to public sector health care is restricted across most of Africa, and even when it is available, health care workers are generally under-trained, under-supplied, poorly-supervised, and/or unmotivated. In response to this crisis, some have advocated for the intentional liberalization of drug access. This strategy is gaining traction in many different countries. Limiting the availability of chemotherapeutic medicines and using them sparingly are two of the most effective ways to slow the spread of drug resistance, and this strategy runs counter to both of those goals. Obviously, a compromise will need to be found to address both the need to increase access to antimalarial medications for people who suffer from the disease and the need to decrease the improper use of these treatments (Salako, 2018).

Malaria preventive strategies may be roughly divided into two categories: those that aim to reduce the prevalence of malaria and those that aim to limit the spread of drug resistance among existing cases. Indirectly affecting the emergence of medicine resistance is the decrease in the number of malaria infections that must be treated and the likelihood that resistant parasites will successfully transfer to additional hosts. Examples include the use of insecticide-treated bed nets, indoor residual spraying of insecticides, pollution monitoring, additional personal protective measures, and chemoprophylaxis in certain groups. It would also be tremendously beneficial if there were a reliable and readily disseminated immunizations available (Salako, 2018).

2.15 Natural Products Used to Treat Malaria

The development of effective antimalarial medications relies heavily on natural ingredients. The immense potential of natural chemicals has been shown, for example, by quinine and artemisinin. These natural products serve as highly effective antimalarials on their own, and also offer valuable lead structures that have served as the basis for creating structurally simplified derivatives. Plants have played a crucial part in the health care of people in malaria-endemic regions for a very long time, and their usage has not diminished. A staggering 80 percent of the African population relies on traditional medicine as their main health care system (World Health Organization (WHO), 2018). Some promising new antimalarial drugs, with unique structures and a probable separate mechanism of action, have emerged in recent years through a number of screens of various natural sources, including plants used locally to treat malaria and marine species. Research on these chemicals has been widely discussed in published articles (Oliveira *et al*., 2019; Turschner and Efferth, 2019).

Several groups of naturally occurring chemicals showed antimalarial activity; the most promising were alkaloids and certain terpenes; specifically, quassinoids and sesquiterpene lactones (Saxena *et al*., 2018).

2.16 *Vernonia amygdalina* (Bitter Leaf)

The tiny shrub *Vernonia amygdalina* (genus Asteraceae) is native to the rainforests of equatorial Africa. For obvious reasons, *V. amygdalina* is also known as bitter leaf. The leaves may be eaten as a vegetable (as macerated leaves in soups) or used to make medicinal aqueous preparations called tonics. Growing up to 23 feet in height, this shrub or

small tree is easily identified by its rough, flaky, grey or brown bark. The blooms are tiny, white, and thistly, and they bloom in dense clusters among the oblong, green leaves. As a result of its fragility, the shrub's branches are often lost. Flowers on bitter leaves are tiny and white; they appear in groups during the wetter months. Small fruits with somewhat hairy nuts are produced by the shrub. The pith, the leaf, and the root have all been attributed healing properties. Bitter Leaf is a popular vegetable in many regions of Africa, where it is served similarly to spinach. You may use the root and the twigs as a starter by chewing them. Tonic, anti-parasitic, anti-tumor, and antibacterial effects have been attributed to bitter leaf. (Lurie, 2017).

2.16.1. Classification of *Vernonia amygdalina*

Bitter leaf plant is classified based on the binomial system of classification below;

Kingdom: Plantae

Order: Asterales

Family: Asteraceae

Genus: *Vernonia*

Species: *amygdalina*

Binomial name: *Vernonia amygdalina* (Zubairu *et al*., 2019).

A **B**

Figure 2.7: *Vernonia amygdalina* in the field (Source: Field survey).

2.16.2. Medicinal uses of *Vernonia amygdalina*

Intestinal parasite infections are treated with bitter leaf. Because many animals in the wild consume this plant when they are unwell, traditional healers may have learned of its healing effects by observing the behaviour of sick animals in the wild (Alara *et al*., 2017).

The traditional medical community often use *Vernonia amygdalina*. Leaf decoctions are used as a laxative, fertility enhancer, and for the treatment of fever, malaria, diarrhoea, dysentery, hepatitis and cough. Medicinal uses include treating scabies, headaches, and stomach aches. In addition to treating malaria and stomach issues, root extracts are employed in a variety of other medicinal applications. The leaves are used in lieu of iodine on wounds in Nigeria. *Vernonia amygdalina* is widely used as an anti-worm medication, especially effective against nematodes. *Vernonia amygdalina* is used by both humans and chimps to treat intestinal worm infections due to its bitter pith. A root infusion is often used

to treat STDs in Zimbabwe. Fever and diarrhoea may be treated with bark infusions, while dried flowers can be used to treat stomach ailments (Fomum, 2019).

The *Vernonia amygdalina* plant is very effective in preventing plant diseases. Seed viability and germination are improved by using ash from burned branches to kill off seed-borne fungus such as *Curvularia, Aspergillus, Fusarium,* and *Penicillium* spp. As an alternative to hop, it has also been included into the brewing process. The *Vernonia amygdalina* plant is well recognised as an important nectar source for honeybees (Fomum, 2019). Both *Plasmodium berghei* and *Plasmodium falciparum*, the two most common forms of malaria, were killed by extracts of the leaves and root bark in a mouse in vivo study. Strong anti-leishmanial activity was also observed in the extracts. *Vernonia amygdalina* wood chewing sticks showed antibacterial activity against periodontal disease-associated microorganisms. The leaves exhibited antimicrobial and antiviral properties (Fomum, 2019).

2.17 *Garcinia kola* (Bitter Kola)

Garcinia kola, often known as bitter kola, is a member of the plant family Guttiferae. It's a woodsy crop that survives year after year, and it may be found throughout Western and Central Africa (Adaramoye *et al*., 2018). Additionally, *G. kola* is widely dispersed across the forested regions of West African nations including Sierra Leone, Ghana, and Cameroon and particularly prevalent in Nigeria's Southwestern and Edo States (Okojie *et al*., 2019). It reaches heights of 15 to 17 metres and has a somewhat thin crown for an evergreen tree of its size. The leaves are simple, measuring between 6 and 14 centimetres in length and 2 and 6 centimetres in width. Red, short hairs cover the surface of the tiny blossoms (Adaramoye *et al*., 2018). The drupe-like fruit has a diameter of 5-10 centimetres and a weight of 30-50 grammes. The pulp within is often a vibrant yellow-red colour and has a silky texture. Each

fruit contains between one and four seeds and ripens from a green to an orange hue (Cowan, 2019).

2.17.1. Classification of *Garcinia kola*

Garcinia kola plant is classified based on the binomial system of classification below;

Kingdom: Plantae

Order: Malpighiales

Family: Clusiaceae

Genus: *Garcinia*

Species: *kola*

Binomial name: *Garcinia kola* (Adaramoye *et al*., 2018).

A **B**

Figure 2.8: *Garcinia kola* plant and seeds

A: *Garcinia kola* plants in the field (Source: Field survey)

B: *Garcinia kola* seeds (Source: Fomum, 2019)

2.17.2 Medicinal uses of *Garcinia Kola*

Tannins, saponins, alkaloids, and cardiac glycosides are only some of the phytochemical substances that have been identified from *G. kola* (Indabawa and Arzai, 2017). Additionally, biflavonoids like kolaflavone and 2-hydroxybi-flavonols were extracted from *G. kola* seeds. In addition to tocotrienol, the isolation of two novel chromanols, garcioic and garcinal, from *G. kola* was also reported (Terashima *et al*., 2020). Multiple traditional applications for the therapy of health issues are supported by the study results on *Garcinia kola*, which reveal that it offers enormous pharmacological value (due to its powerful phytoconstituents: kolaviron and GB-1). Studies conducted by Damian *et al*. (2017) and Oluwatosin *et al*. (2019) evaluated the effectiveness of the biflavonoid fraction from *Garcinia kola* seeds (kolaviron), against *Plasmodium berghei* infection in Swiss albino mice. In mice infected with *Plasmodium berghei*, kolaviron showed potent antimalarial actions in addition to its well-documented antioxidant benefits, particularly at 200 mg/kg. At the levels used, it also reduced the anaemia and weight loss caused by the parasite, probably by blocking the lipid peroxidation process and protecting the body's stores of key antioxidant enzymes. Another research carried out by Konziase, 2015 found that the three pure biflavanones (GB-1a, GB-1, and GB-2) isolated from the same plant had in-vitro and *in-vivo* antimalarial potencies. Indeed, they showed not only antimalarial efficacy by oral treatment in mice infected with *Plasmodium berghei*, but also significant inhibitory effect in-vitro against *Plasmodium falciparum* growth. Since GB-1 had the highest levels of antimalarial activity and selectivity index, it might be used to help pinpoint the molecular basis, which in turn could be used to the development of new treatments for malaria. It's

possible that GB-1 might be a good antimalarial candidate worthy of testing in infected higher animals.

2.18 *Cymbopogon citratus* (Lemon Grass)

The plant *Cymbopogon citratus*, sometimes known as citronella or lemon grass, is a member of the Gramineae family. It is a tall perennial grass that is grown for its intense fragrance in many regions of Africa, particularly Nigeria. It is also widely farmed in the West Indies, North and South America, and tropical Asia. It is a major cash crop in many parts of the tropics and subtropics. Plants thrive in full sun and warm, humid conditions on well-drained sandy soils (Prakash and Rao, 2017). About 0.4% of the fresh weight of *C. citratus* grass is volatile oil. Citral, a combination of the geometric isomers geraniol and neral, makes up anywhere from 65% to 85% of the oil. Nerolide, geraniol, and geranic acid, all of which are related chemicals, have also been isolated (Abe *et al*., 2018; Naik *et al*., 2019). In addition to the myrcene (12-25% of the oil), the oil contains a wide variety of other compounds, including diterpenes, luteolins, methylheptenone, linalool other alcohols, aldehydes, farnesol, terpineol, beta sitosterol, citronellol, coumarin, tannin, alpha pinene, usorlic acid, and a few dozen other minor fragrant components (Calixto, 2020). *Cymbopogon citratus* has several non-volatile components, such as caffeic acid, luteolins, homo-orientin, cP-coumaric acid, hlorogenic acid, luteolins, fructose, sucrose, octacosanol, and others.

2.18.1. Classification of *Cymbopogon citratus*

Cymbopogon citratus plant is classified based on the binomial system

Kingdom: Plantae

Order: Poales

Family: Poaceae

Genus: *Cymbopogon*

Species: *citratus*

Binomial name: *Cymbopogon citratus (DC.)* (Naik *et al*., 2019).

A

B

Figure 2.9: *Cymbopogon citratus* plants. (Source: Elujoba *et al.,* 2018).

2.18.2 Medicinal uses of *Cymbopogon citratus*

To alleviate the agony of a headache, many people turn to lemon grass, which has been shown to be an excellent painkiller. This action may be traced back to the essential oil myrcene, which also validates the herb's traditional usage in Brazil as a pain reliever. Muscle cramps, rheumatism, and spasms are among things that the plant is known to help with (Elujoba *et al*., 2018).

Myrcene, a component of lemon grass, is a powerful relaxant and moderate sedative that helps individuals who are stressed out and have high blood pressure. This confirms the herb's traditional sedative usage in Brazil, and it's also used to treat insomnia (Okigbo and Ajalie, 2018).

Due to its astringent characteristics, lemon grass is used to treat colds, sore throats, and the flu (particularly with headaches and fevers) (Naik *et al*., 2019).

Herbalists often use lemon grass as a tonic and supplement to aid in clearing blemishes and maintaining a balanced skin tone. Researchers are investigating if lemon grass has anti-mutagenic effects. In recent years, myrcene's ability to mitigate toxicity and mutagenesis consequences has been established (Vaziriana *et al*., 2017). Lemon grass, which is high in geraniol and citral, may help decrease blood cholesterol. A possible mechanism of action involves blocking an enzyme process, so preventing cholesterol from being synthesized from less complex fatty acids. The plant is also supposed to treat headaches, bring down fluctuating temperatures, and clear mucus from the lungs (Naik *et al*., 2019).

2.19 *Tetracarpidium conophorum* (African Black Walnut)

Walnuts are a popular nut because of its tasty and nutritious seeds. Most notably, the English walnut (*Juglans regia*), is a member of the family Juglandaceae and is one of the most well-known species in this genus (Burkill, 2018). In the family Euphorbiaceae lives the tropical African walnut, also called *Plukenetia conophora* or *Tetracarpidium conophorum* (Oyekale *et al*., 2019).

Gawudi bairi [Hausa] is the name given to it in the northern part of Nigeria. The cities of Akamkpa, Lagos, Uyo, Akpabuyo, Kogi, Ogbomoso, and Ibadan are all home to

Tetracarpidium conophorum in Nigeria (Ayoola *et al*., 2018). Mainly the nuts, which may be eaten raw or cooked, are the reason this plant is planted. The nut has a high dietary energy value and is a great protein source. In length, the seed/nut is around 25 mm (0.98 in). Single-shelled nuts, double-shelled nuts, and even three-shelled nuts may all be found in the same pod. The plant's walnuts may have either a black or brown hull. After being removed from the shell, the nut reveals a white colour inside. A thin membrane separates the nut's two halves (Ayoola *et al*., 2018). The seeds may be purchased at your neighbourhood farmers' market between the summer months and September, when they have matured for a period of 4 to 6 months. When boiled, the nut is a popular snack in Nigeria (Nwaoguikpe and Ujowundu, 2019).

2.19.1. Classification of *Tetracarpidium conophorum*

Tetracarpidium conophorum plant is classified based on the binomial system of classification below;

Kingdom: Plantae

Order: Euphobiales

Family: Euphobiaceae

Genus: *Tetracarpidium*

Species: *conophorum*

Binomial name: *Tetracarpidium conophorum* (Dada and Ogundolie, 2019).

A **B**

Figure 2.10: *Tetracarpidium conophorum* mature pod (A) and nuts (B)

Source: Ayoola *et al*. (2018).

2.19.2 Medicinal uses of *Tetracarpidium conophorum*

The raw seed extract of *T. conophorum* exhibited a dose-dependent impact on suppression in Swiss albino mice infected with the parasite *Plasmodium berghei*, according to research by Dada and Ogundolie, (2019) on the efficacy of *T. conophorum* against malaria. Exactly 600 mg/kg produced maximal suppression (47.22%), whereas 5 mg/kg of chloroquine produced the greatest level of suppression (55.50%). Results like this lent credence to the idea also that seed nut may be useful as a malaria treatment. Studies on the effects of raw alcoholic extract seed leaves extract conophorum on the hematological parameters and morphological characteristics of Swiss albino mice activity against *P. berghei* have yielded similar findings (NK65). All treatment groups showed a rise in packed cell volume (PCV), erythrocyte count (EC), hematocrit (HGB), and platelet count (PLT). Liver and kidney

function were recovered in mice given 250 and 500 mg of the seed extract, whereas animals administered 600 mg/kg of said essential oil had their kidney function restored but their liver function harmed, as determined by histology. The researchers, Ogundolie *et al*. (2017) concluded that *T. conophorum* stem extract at a concentration of 400 mg/kg would be effective in treating patient communicable disease.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in Minna, Niger State, North Central, Nigeria. Coordinates: 9.5836^0N 6.5463^0E. It has a total of 29,484 square metres (76,363 km square), and a population of 304,113 which consists majorly of two ethnic groups (Nupe and Gwari).

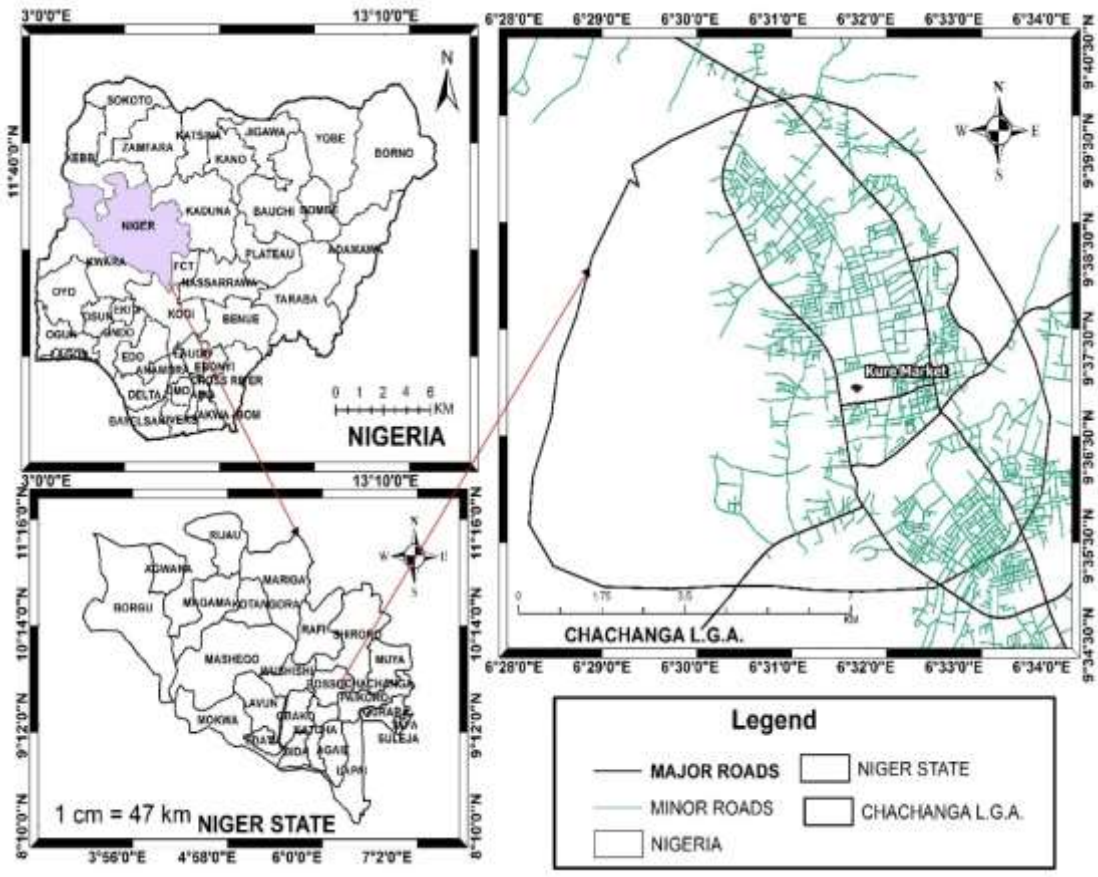


Figure 3.1: Map of Study Area, Chanchaga L.G.A. Niger State.

Source: Department of Geography, Federal University of Technology, Minna, Niger State.

3.2 Materials

3.2.1 Reagents and chemicals

All chemicals and reagents used were products of Sigma Chemical Company in the United States, and were of analytical quality.

3.2.2 Plant samples (collection and identification)

Bitter kola (*Garcinia kola*) and African walnut (*Tetracarpidium conophorum*) were bought in a market in Minna, Niger State. Bitter leaf (*Vernonia amygdalina*) and lemon grass (*Cymbopogon citratus*) were gathered at Airforce base Minna in Niger State, Nigeria. All of these were recognized and confirmed by experts in the Biological Sciences Department at the Federal University of Technology in Minna, Niger State, Nigeria.

3.3 Sample Processing

Two kilograms of Bitter kola and African walnut to be de-hulled, rinsed in distilled water, washed again, cut into tiny bits with a stainless-steel knife, and air-dried at room temperature for a month. A Silver Crest 5000W Blender was used to grind them into a powder after they had first been reduced to small bits using a mortar and pestle. Before analysis, the powder was sealed in an airtight container (Damian *et al*., 2017 and Sampson *et al*., 2019).

Lemon grass and bitter leaf leaves weighing two kilograms were washed in distilled water, drained, and dried in the open air for two weeks. The leaves were crushed using a crusher and pestle into fine powder, then kept in an airtight container until further examination could be performed (Ekpo and Ekanemesang, 2016).

3.4 Extraction Procedure

Extracts were made using the same procedure described by Ekpo and Ekanemesang, (2016), Damian *et al*. (2017), and Sampson *et al.* (2019). Exactly 1 kg of powdered Bitter kola, African walnut, Lemon grass and Bitter leaf was weighed out and split into half (each half weighing 500g) before dividing it evenly between 2 conical flasks. A total of four litres of 95% ethanol was poured into one of the flasks until the sample was fully covered. The other flask had four litres of distilled water added to it until the sample was fully buried.

To maximize extraction, the initial 500 g of powder were macerated in the solvent (ethanol) for 24 hours in a rotating shaker. The second 500g of powder was also macerated in distilled water for 24 hours using a rotary shaker. In order to separate the two mixes, filter paper was used. At a temperature of 40 degrees Celsius, the ethanol and distilled water were allowed to evaporate in a rotating evaporator. Using a lyophilizer (FREEZE-DRYER)-LGJ-18, the concentrated extracts were dehydrated. The extracts were kept cold, at 4^0C. The extract yield % was calculated using Equation 3.1.

$$\text{Extract yield (wt \%)} = \frac{\text{Weight of obtained extract}}{\text{Weight of powdered sample}} \times 100 \quad (3.1)$$

3.5 Phytochemical Screening

Extracts of medicinal plants were analyzed qualitatively and quantitatively for their bioactive contents (phenols, anthraquinones, alkaloids, terpenoids, glycosides, saponins, flavonoids, tannins, steroids, and) using standard protocols described by Rehab and Saad, (2016).

3.5.1 Test for phenols

To identify phenolic chemicals, 2 mL of 1% ferric chloride was added to 2 mL of water and ethanol extracts in distinct tubes and then 2 mL of potassium ferro-cyanide to each. The presence of phenols was confirmed by the development of a bluish-green tint (Sampson *et al*., 2019).

3.5.2 Test for free anthraquinone

Three millilitres of the water and ethanol extracts were placed in individual test tubes, and five millilitres of the 10% potassium hydroxide solution were added and stirred in. If a red precipitate appeared during an anthraquinone test, the result was affirmative (Sampson *et al*., 2019).

3.5.3 Test for saponins (Foam test)

Each extract was diluted to 3 ml with distilled water, and the tube was stirred for roughly 10 minutes to mix the ingredients. The presence of saponins was shown by the appearance of lather or froth on top (Arome *et al*., 2016).

3.5.4 Test for terpenoids

Each extract was diluted to a level of 3 mL using Chloroform, and then 3 mL of concentrated H_2SO_4 was poured to the bottom of the vial. A reddish-brown coloration on the surface indicated the possible presence of terpenoids (Sampson *et al*., 2019).

3.5.5 Test for flavonoids

A two drops of diluted sodium hydroxide were added to the extract solution to bring it to a neutral pH. The formation of a vivid yellow colour that disappeared after being treated with

a few drops of diluted sulfuric acid revealed the existence of flavonoids. This has been shown by many researchers (Arome *et al*., 2016).

3.5.6 Test for tannins (Ferric chloride test)

After dissolving each extract in 10 mL of distilled water, the mixture was filtered to remove any impurities. The filtrate was then treated with a few drops of a 0.1% Ferric chloride solution. The presence of tannin was shown by the formation of a blue-black precipitate (Sampson *et al*., 2019).

3.5.7 Test for alkaloids

To one gram of each extract, 3 millilitres of ammonia were put in individual test tubes. A few minutes of standing time was given to them. Then, 10 mL of chloroform was added, and the mixture was agitated before being filtered. A water bath was used to evaporate chloroform, and then Mayer's reagent was added. There was evidence of alkaloids due to the presence of a cream-colored precipitate (Arome *et al*., 2016).

3.5.8 Test for glycosides

A total of 1 mL of glacial acetic acid was added to 2 ml of each extract solution before being spiked with 6 drops each of 10% ferric chloride solution and concentrated sulfuric acid. The inclusion of glycosides was shown by the creation of a greenish-blue hue (Arome *et al*., 2016).

3.5.9 Test for steroids

Each extract was dissolved in 1gram of chloroform in 10 millilitres of chloroform, and 1 millilitre of strong sulfuric acid was added to the test tube. Red pigmentation was seen in

the top layer, indicating the presence of steroids, while yellow pigmentation was observed in the sulfuric acid layer (Arome *et al*., 2016).

3.6 *In-vivo* Assay

3.6.1 Collection and grouping of experimental mice

About 100 albino mice weighing 12–23 grams were purchased from an animal facility in Vom, Plateau State. Mice were kept at the animal house at the Federal University of Technology, Minna, where they were provided with pellets and water ad-libitum. Acclimatization to room temperature took place over the course of 7 days. Mice used in the experiments were split into five groups of four. Group A was treated with Arthemether+Lumefantrine as a positive control, while Group B was left untreated. Extracts from different plants were tested in Groups C, D, and E for their ability to inhibit parasite multiplication.

Table 3.1: Grouping of Experimetal Mice

Experimental Group of Mice	Number of Mice	Treatment	Purpose
A	4	arthemether+Lumafantrine	Positive Control
B	4	Not Treated	Negative Control
C	4	Plant Extract	Antiplamodial activity
D	4	Plant Extract	Antiplamodial activity
E	4	Plant Extract	Antiplamodial activity

Source: Ogundolie *et al*. (2017)

3.6.2 Acute toxicity test and determination of safe dose

According to Olaniyi *et al.* (2016) description of the Lorkes technique, lethal dosage (LD_{50}) of plant extracts were determined. For this acute toxicity study, a total of 28 mice were

employed. The mice were arbitrarily split into seven groups of four. Both before and after the injection of the extracts, all mice were fasted for a total of 6 hours. To begin, three groups of mice were given oral administration of extracts at dosages of 10 mg/kg, 100 mg/kg, and 1000 mg/kg. In phase 2, three further groups of mice were given oral administration of extracts at dosages of 1900 mg/kg, 3600 mg/kg, and 5000 mg/kg. Finally, a control group of mice received 20 millilitres of normal saline (0.9%w/v Nacl) per kilogram of body weight. Twenty-four hours of observation revealed reduced activity, paw licking, body weakening, sleep, and death in the mice. The effective extract dose was calculated as the intersection of the % mortality (y axis) and doses (x axis).

3.7 Collection of Parasites

At the Nigerian National Institute for Pharmaceutical Research and Development (NIPRD) in Abuja, a malaria parasite strain (*Plasmodium berghei* NK 65) in a mouse that is sensitive to chloroquine was obtained. Parasites were maintained by injecting 0.2 millilitres of parasitized blood from a donor mouse into the peritoneal cavity of a recipient mouse. This freshly infected mouse had 0.2 ml of its parasitized blood removed through heart puncture and serially diluted with 4.8 ml of sterile normal saline. It was diluted to 0.2 millilitres and administered to the mice in groups A, B, C, D and E. Mice were monitored visually for signs of altered behaviour (decreased activity, loss of appetite, sleeping) (Yerbanga *et al*., 2016).

3.8 Determination of Body Weight and PCV of Experimental Mice

Every mouse in every group was weighed and their blood was tested before, during, and after an acute toxicity test, an infection, and a series of treatments (Dada and Muhammed, 2018). The PCV was measured by drawing blood from the mouse into a capillary tube and analyzing it with a hematocrit reader. A precise digital weighing scale was used to calculate

their body masses. Equation 3.2 was used to determine the average weight of the participants.

$$\text{Mean Body Weight} = \frac{\text{Total weight of mice in a group}}{\text{Total number of mice in that group}} \quad (3.2)$$

3.9 Administration of Extracts and Standard Drug

Extracts were made to create varied concentrations (100, 200, 400 mg/kg body weight) and given orally as treatment dosages to mice in groups C, D, and E after 3 days of *Plasmodium berghei* infection. The mice in Group A (the positive control) were given 5 mg/kg of arthemether+lumafantrine in 0.2 mL of solution, whereas the animals in Group B (the negative control) were not given any medication. To maximize their effectiveness, the therapies were given over the course of seven days (Ogundolie *et al.*, 2017).

3.10 Determination of Parasitaemia

The mice's parasitemia levels were checked once before treatment and once a day for seven days straight. The mice's parasitemia was measured by placing a drop of blood from a venesection of the tail onto a clean glass slide. Blood was smeared thinly and allowed to dry at room temperature. It was stained for 10 minutes with a Giemsa stain concentration of 10%, then flooded with water after being fixed with methanol. After letting the slides air dry, they were seen using a light microscope at a magnification of X100 (oil immersion). At the same time, a thick blood smear was made and left to dry at room temperature (25^0C). A 10% Giemsa stain was applied for 10 minutes, and then the area was soaked with water. allowed to dry at room temperature before being inspected under a microscope (oil immersion).

By counting the number of parasites present, the prevalence of parasitaemia was determined. Parasite count divided by white blood cell (WBC) count (200 WBC/200

parasites) multiplied by 8000 (Idowu *et al*., 2018). Using the Equation 3.3, the parasite density was compared in infected groups to those who received various concentrations of the test extract and the average % parasite suppression for each test concentration were determined using Equation 3.4

$$\text{Parasite Density (µl)} = \frac{\text{Number of Parasite}}{200\ \text{WBC}} \times 8000 \qquad (3.3)$$ (Idowu *et al.,* 2018)

$$\text{Average \% parasite suppression} = \frac{(\text{Parasitaemia in negative control} - \text{Parasitaemia in treated}) \times 100}{\text{Parasitaemia in negative control}}$$

(3.4) (Ogundolie *et al.,* 2017)

3.11 Determination of Mean Survival Time (MST)

The impact of the plant extracts on the survival of *P. berghei*-infected mice was measured by keeping track of the number of days the mice lived. Mice were allowed free access to food and water and studied for roughly 30 days. During this time, the survival rate of each mouse in the treatment and control groups was tracked and the mean survival time was recorded. The average number of days' mice in each group survived after infection, expressed as a number, was calculated using Equation 3.5 to determine their mean survival time (MST) at the end of the study period.

$$\text{MST} = \frac{\text{Sum of Survival Time of all mice in each group (Days)}}{\text{Total number of mice in that group}} \qquad (3.5)$$ (Chaniad *et al.,* 2019)

3.12 Haematological Analysis

To evaluate how plant extracts and *P. berghei* affected the haematological parameters in the experimental mice, this study was conducted. Blood counts were taken to determine the percentages of red blood cells, white blood cells, platelets, packed cell volume,

haemoglobin concentration, mean cell haemoglobin concentration, mean cell corpuscular volume, and mean cell haemoglobin as well as the numbers of lymphocytes, neutrophils, monocytes, and eosinophils. The mice were euthanized with chloroform, dissected, and their blood was taken through heart puncture into ethylene diamine tetraacetic acid (EDTA) bottles for later analysis using an Abacus 380 Auto haematological analyzer on day 7 (Ogundolie *et al*., 2017).

3.13 Histopathological Studies

Histopathological study was performed using the procedure described by Ogundolie *et al*. (2017) to assess the impact of the plant extracts and parasite on the kidneys and livers of mice. Mice were euthanized using chloroform, and their organs were removed and preserved in 10% formalin for 48 hours. The oxidizing chemicals in the fixative were removed by repeatedly washing the organs in running water. After slicing the organs, they were hung in the decalcifying solution using a waxed thread. After the tissues had been decalcified, they were placed in 70% alcohol for 4 hours, then 90% alcohol for 4 hours, and lastly absolute alcohol for 4 hours over 2 changes. After 1 1/2 hours of cleaning in xylene, the tissues were transferred to a bath of molten paraffin wax in a mould using a warm pair of blunt-nosed forceps.

A small layer of wax was blasted over the surface of the mould before it was submerged in cold water for 30 minutes to harden. The block containing the implanted tissues was trimmed and then sectioned using a microtome machine. The resulting ribboning was carefully applied to the surface of a water bath maintained at a comfortable 10 degrees Celsius.

When the tissue sectioning had reached its full length, a clean, grease-free slide was dipped at an angle into the water such that its surface touched the edge of the section. Using a mounted needle, each slide was carefully withdrawn and repositioned to connect to the desired location.

Slides containing sectioned tissue were placed on a hot plate set to 45°C for 1 hour to speed up the drying process before staining. For 30 minutes, slides were submerged in xylene to remove paraffin wax before being stained. After 30 seconds in xylene-removing absolute alcohol, the slides were re-examined. The slide was handled carefully with blunt-nosed forceps as it was placed in 90% and 70% alcohol for 30 seconds each before being cleaned completely in distilled water.

The slides were removed after letting solution one soak into the slides for 30 minutes. They were cleaned in running water and then put through a differentiation process in solution two until no more colour remained. Solution 3 was used to immerse the slides in scotts' tap water for 10 minutes, and solution 4 (Eosine) was used to counterstain the slides for 2 minutes. After that, they were rinsed under running water to eliminate any remaining traces of eosin. They spent 30 seconds each in 70%, 90%, and absolute alcohol before being dehydrated, and then 3 minutes clearing with xylene. Mounting the slides with Canada balsam and cover slip. Mice were examined microscopically to see whether there were any differences in the morphology of their tissues between uninfected and infected mice (Ogundolie *et al*., 2017).

3.14 Data Analysis

The data generated were subjected to statistical analysis performed using a one-way analysis of variance (ANOVA). When significant differences were detected, the differences

among the mean values were determined using Duncan's multiple comparison test at a confidence level of $p<0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. Results

4.1.1 Yield of plant extracts

Aqueous and ethanol solvents were used to extract the four plants used in this investigation. Weights and production percentages for each extract per plant are shown in Table 4.1. *Garcinia kola* ethanolic extract yielded 13.96%, *Tetracarpidium conophorum* extract yielded 11.58%, and *Cymbopogon citratus* extract yielded 1.24%.

Table 4.1: Yield of Plant Extracts

Plant material/1000g	Solvent	Extract weight (g)	% Yield
Bitter Leaf	Aqueous	16.71	3.34
	Ethanol	13.12	2.62
Bitter Kola	Aqueous	23.00	4.60
	Ethanol	41.89	13.96
Lemon Grass	Aqueous	9.06	1.81
	Ethanol	6.21	1.24
African Walnut	Aqueous	40.57	8.11
	Ethanol	57.92	11.58

4.1.2 Phytochemical constituents of the plant extracts

Saponins, alkaloids, tannins, phenols, and tannins were present in all the aqueous and ethanol extracts, but steroids, terpenoids, and anthraquinones were mostly lacking, as shown by the qualitative phytochemical composition of plant extracts (Table 4.2).

Table 4.2: Qualitative Phytochemical Composition of Aqueous and Ethanolic Plant Extracts

Crude extracts	**Bitter kola** (*Garcinia kola*)		**Bitter leaf** (*Vernonia amygdalina*)		**Walnut** (*Tetracarpidium conophorum*)		**Lemon grass** (*Cymbopogon citratus*)	
	Aqueous	**Ethanol**	**Aqueous**	**Ethanol**	**Aqueous**	**Ethanol**	**Aqueous**	**Ethanol**
Phenols	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+
Alkaloid	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+
Steroid	-	+	-	+	+	-	-	-
Cardiac glycosides	+	+	+	+	+	+	+	+
Terpenoids	+	-	-	+	+	+	-	+
Anthraquinone	-	+	+	+	-	-	-	+

+: Present

-: Not detected

4.1.2.1 Quantitative phytochemical composition of the aqueous and ethanolic plant extracts of bitter kola **(*Garcinia kola*)**

Tannins were present in high quantity in both the ethanol and aqueous extracts of Bitter kola (*Garcinia kola*), as shown by quantitative phytochemical analysis. In the aqueous and ethanol extracts, phenols came in second (8.10±0.10 mg/g) and third (7.78±0.02 mg/g). Then, the Alkaloids concentrations in the ethanol and water extracts were 4.06±0.01 mg/g and 2.11±0.01 mg/g, respectively (Table 4.2b).

4.1.2.2 Quantitative phytochemical composition of the aqueous and ethanolic plant extracts of african walnut **(*Tetracarpidium conophorum*)**

According to quantitative phytochemical analysis, saponins (16.83±0.30 mg/g) were shown to be the most abundant phytochemical in African Walnut (*Tetracarpidium conophorum*) ethanol extracts. However, the maximum quantity of Flavonoids was found in the aqueous extracts, at 10.22±0.10 mg/g. There were moderate quantities of phenolics in both the aqueous and ethanol extracts (4.53±0.02 mg/g and 9.12±0.20 mg/g) (Table 4.2b).

4.1.2.3 Quantitative phytochemical composition of the aqueous and ethanolic plant extracts of bitter leaf **(*Vernonia amygdalina*)**

The quantitative phytochemical analysis of the Bitter Leaf (*Vernonia amygdalina*) extract showed the presence of phenols (184.86±0.48 mg/g and 103.47±1.32 mg/g) in both the ethanolic and aqueous extracts respectively high concentration. This was followed by tannins (87.43±0.26 mg/g and 39.50±0.37 mg/g) both in the ethanol and aqueous extracts. Then flavonoids (3.65±0.05 mg/g and 3.95±0.02 mg/g) both in the ethanol and aqueous extracts respectively (Table 4.2b).

4.1.2.4 Quantitative phytochemical composition of the aqueous and ethanolic plant extracts of lemon grass **(*Cymbopogon citratus*)**

The quantitative phytochemical analysis of the Lemon Grass (*Cymbopogon citratus*) extract showed the presence of phenols (12.75±0.05 mg/g) in the ethanol extracts in the highest concentration. However, the presence of alkaloid (6.78±0.01 mg/g) was recorded as the highest concentration in the aqueous extracts. In both solvent of extraction, saponins (0.35±0.01 mg/g), (0.42±0.01 mg/g) in the aqueous and ethanol extracts respectively were detected in low concentrations (Table 4.2b).

Table 4.2b: Quantitative Phytochemical Composition of Aqueous and Ethanolic Plant Extracts

Plant Extracts	Phytochemicals									
	Aqueous					Ethanolic				
	Ph	Fl	Ta	Alk	Sa	Ph	Fl	Ta	Alk	Sa
Bitter Kola	8.10±0.10^{d}	7.51±0.02^{c}	8.96±0.01^{e}	2.11±0.01^{a}	2.69±0.02^{b}	7.78±0.02^{c}	10.21±0.02^{d}	11.02±0.02^{e}	4.06±0.01^{a}	4.87±0.01^{b}
African Walnut	4.53±0.02^{b}	10.22±0.10^{d}	0.45±0.01^{a}	0.38±0.03^{a}	5.02±0.01^{c}	9.12±0.20^{c}	1.17±0.01^{a}	2.81±0.01^{b}	2.30±0.01^{b}	16.83±0.30^{d}
Bitter Leaf	103.47±1.32^{e}	3.95±0.02^{a}	39.50±0.37^{c}	25.37±0.46^{b}	90.04±0.29^{d}	184.86±0.48^{e}	3.65±0.05^{a}	87.43±0.26^{c}	29.67±1.07^{b}	122.46±0.63^{d}
Lemon Grass	4.30±0.01^{d}	3.21±0.01^{c}	1.69±0.01^{b}	6.78±0.01^{e}	0.35±0.01^{a}	12.75±0.05^{e}	5.47±0.04^{b}	7.04±0.02^{c}	7.52±0.11^{d}	0.42±0.01^{a}

Ph: Phenol; Fl: Flavonoid; Ta: Tannin; Alk: Alkaloid; Sa: Saponin

Mean± Standard error. Similar values in the same row that have the same superscript are not statistically different ($P<0.05$) at the 5% level.

4.1.3 Acute toxicity (LD_{50}) of the aqueous and ethanolic plant extracts in albino mice

Table 4.3 shows the results for the determination of acute toxic dose (LD_{50}) of the plant extracts in albino mice. The administration of doses in phase 1 ranged from 100 to 1000 mg/kgbw and phase 2 of the toxicity test ranged from 1900 to 5000 mg/kgbw of both the aqueous and ethanolic plant extracts. There were no accompanied physical and behavioral changes neither was any mortality recorded. These results show that the LD_{50} of the plant extracts could be greater than 5000 mg/kgbw.

Table 4.3: Acute Toxic Dose of Aqueous and Ethanolic plant extracts in Albino mice

	Group	**Dosage (mg/kgbw)**	**Observation**	**Mortality/No. of Mice**
Phase 1	1	10	-	0/4
	2	100	-	0/4
	3	1000	-	0/4
Phase 2	4	1900	-	0/4
	5	3600	Sleeping	0/4
	6	5000	Sleeping	0/4

mg/kgbw: milligram per kilogram body weight

-: No apparent changes.

4.1.4 Effect of aqueous and ethanolic plant extracts on body weight of *P. berghei*-infected mice

Weight loss was seen in the infected, untreated mice group, whereas weight gain was observed in the infected, plant extract-treated mice group. After receiving either the aqueous or ethanolic extracts, mice infected with *P. berghei* gained significantly more weight than they had before therapy. As shown in table 4.4, at the end of both the aqueous

and ethanolic extracts administration, there was significant weight gain in the aqueous extract of bitter leaf and lemon grass than the extracts of walnut and Bitter kola.

Table 4.4: Body Weight Variation in *P. berghei*-infected mice Treated with Aqueous and Ethanolic Plant Extracts.

Crude Extracts	Dose	Aqueous extract		Ethanolic extract	
	(mg/kgbw)	Initial weight	Final weight	Initial weight	Final weight
Bitter leaf	100	14.17 ± 0.01^{b}	25.23 ± 0.01^{i}	15.77 ± 0.01^{e}	19.64 ± 0.02^{d}
	200	20.26 ± 0.01^{j}	27.85 ± 0.02^{m}	16.17 ± 0.02^{f}	22.20 ± 0.02^{h}
	400	17.06 ± 0.03^{h}	25.03 ± 0.02^{h}	12.81 ± 0.13^{a}	20.17 ± 0.02^{e}
Walnut	100	15.66 ± 0.03^{d}	21.36 ± 0.02^{d}	17.67 ± 0.02^{1}	20.12 ± 0.01^{e}
	200	20.34 ± 0.04^{k}	24.44 ± 0.02^{f}	15.84 ± 0.03^{e}	21.12 ± 0.02^{g}
	400	14.07 ± 0.01^{a}	23.53 ± 0.03^{e}	14.72 ± 0.02^{d}	17.02 ± 0.02^{c}
Bitter kola	100	15.44 ± 0.04^{a}	18.27 ± 0.01^{b}	14.26 ± 0.01^{c}	17.06 ± 0.03^{c}
	200	23.03 ± 0.03^{m}	25.84 ± 0.02^{j}	13.83 ± 0.03^{b}	15.27 ± 0.02^{b}
	400	16.02 ± 0.02^{g}	20.23 ± 0.03^{c}	17.56 ± 0.03^{i}	21.04 ± 0.01^{f}
Lemon grass	100	15.84 ± 0.02^{e}	24.72 ± 0.02^{g}	19.25 ± 0.02^{l}	24.23 ± 0.03^{j}
	200	20.08 ± 0.01^{i}	26.24 ± 0.01^{k}	17.02 ± 0.01^{h}	20.14 ± 0.02^{e}
	400	21.07 ± 0.02^{l}	26.73 ± 0.02^{l}	16.33 ± 0.01^{g}	22.19 ± 0.01^{h}
Positive Control (Arthemether + Lumafantrine)	5	15.87 ± 0.02^{e}	28.53 ± 0.01^{n}	17.84 ± 0.02^{j}	23.52 ± 0.02^{i}
Negative Control	--	23.02 ± 0.02^{m}	16.56 ± 0.02^{a}	18.84 ± 0.05^{k}	12.72 ± 0.02^{a}

Mean± Standard error. Similar values in the same row that have the same superscript are not statistically different ($P<0.05$) at the 5% level.

4.1.5 Effect of combined ethanolic plant extracts on the body weight of *P. berghei*-infected mice

Mice infected and treated with 400mg/kg combined extract of bitter kola and bitter leaf showed a loss in body weight (27.31g - 24.91g). Mice infected and not treated (negative control) also showed decrease in body weight at the end of the treatments. There was significant weight gain after treatment in *P. berghei*-infected mice with combined extracts of Bitter leaf and Lemon grass (27.55g - 31.02g); and Bitter leaf and Walnut (29.51g - 31.17g) extracts (Table 4.5).

Table 4.5: Body Weight Variation in *P. berghei*-Infected Mice Treated Combined Ethanolic Plant extracts.

Crude Extracts	Dose (mg/kgbw)	Body Weight (g)	
		Initial weight	Final weight
Bitter leaf + Bitter Kola	400	27.31±0.02 [b]	24.91±0.01 [b]
Bitter leaf + Walnut	400	29.51±0.02 [e]	31.17±0.01 [d]
Bitter leaf + Lemon grass	400	27.55±0.01 [c]	31.02±0.02 [c]
Positive Control (Arthemether + Lumafantrine)	5	26.53±0.01 [a]	35.23±0.03 [e]
Negative Control	--	27.92±0.02 [d]	20.19±0.01 [a]

Mean ± Standard error. Similar values in the same row that have the same superscript are not statistically different ($P<0.05$) at the 5% level.

4.1.6 Effect of aqueous plant extracts on the packed cell volume (PCV) in *P. berghei*-infected mice

The PCV of *Plasmodium berghei*-infected mice treated with increasing concentrations of the aqueous crude extracts is shown in Table 4.6. The PCV dropped in the contaminated and treated group both after infection and after treatment. The reduction in PCV (30.7±0.02-20.5±0.02) recorded in the negative group was statistically significant, in contrast to the rise in PCV (39.40.02- 46.10.02%) observed in the group treated with the control medication.

Table 4.6: Effect of Aqueous Plant Extracts on the Packed Cell Volume (PCV) of *P. berghei*-Infected Mice.

Crude Extracts	Dose (mg/kgbw)	PCV(%)		
		Before Infection	Treatment at Day 1	Treatment at Day 7
Bitter leaf	100	40.35±0.15 ^{def}	37.4±0.20 ^{f}	35.2±0.15 ^{g}
	200	40.9±0.10 ^{fg}	37.7±0.15 ^{f}	38.9±0.10 ^{j}
	400	39.9±0.05 ^{cde}	35.8±0.10 ^{e}	30.1±0.05 ^{d}
Walnut	100	43.5±0.15 ^{h}	39.8±0.10 ^{h}	38.2±0.15 ^{i}
	200	40.9±0.55 ^{fg}	31.5±0.05 ^{c}	42.3±0.25 ^{k}
	400	37.9±0.25 ^{a}	35.3±0.05 ^{d}	31.2±0.20 ^{e}
Bitter kola	100	40.7±0.35 ^{ef}	38.5±0.25 ^{g}	29.3±0.25 ^{c}
	200	39.7±0.25 ^{bcd}	29.5±0.10 ^{a}	19.8±0.20 ^{a}
	400	37.5±0.40 ^{a}	35.1±0.05 ^{d}	25.2±0.15 ^{b}
Lemon grass	100	39.8±0.20 ^{cde}	37.7±0.15 ^{f}	32.1±0.05 ^{f}
	200	40.1±0.20 ^{cdef}	39.4±0.05 ^{h}	38.2±0.15 ^{i}
	400	38.9±0.20 ^{b}	38.4±0.15 ^{g}	36.3±0.25 ^{h}
Positive control (Arthemether + Lumafantrine)	5	41.7±0.20 ^{g}	39.4±0.16 ^{h}	46.1±0.10 ^{l}
Negative control		39.5±0.25 ^{bc}	30.7±0.30 ^{b}	20.5±0.05 ^{a}

Mean± Standard error. Similar values in the same row that have the same superscript are not statistically different (P<0.05) at the 5% level.

4.1.7 Effect of ethanolic plant extracts on the packed cell volume (PCV) of *P. berghei*-infected mice

Overall packed cell volume (PCV) of mice that were afflicted with *Plasmodium berghei* and then treated with various dosages of ethanolic plant extracts is shown in Table 4.7. These animals were then tested for their ability to fight off the malaria parasite. The PCV was found to have dropped both after infection and after treatment in all of the groups. This was shown to be the case in the infected and treated group. When compared to the pretreated with the control treatment, which led to a rise in the PCV (39.6–47.4%), the PCV decreased significantly in the negative group, where it was reported to decline from 32.7 to 29.7%.

Table 4.7: Effect of Ethanolic Plant Extracts on the Packed Cell Volumes (PCV) of *P. berghei*-Infected Mice.

Crude Extracts	Dose (mg/kgbw)	PCV(%)		
		Before Infection	Treatment at Day 1	Treatment at Day 7
Bitter leaf	100	42.2±0.10 ^{g}	35.9±0.10 ^{gh}	28.6±0.20 ^{d}
	200	41.3±0.10 ^{f}	34.3±0.15 ^{d}	29.8±0.05 ^{e}
	400	41.9±0.20 ^{g}	37.2±0.15 ^{i}	31.8±0.10 ^{g}
Walnut	100	36.4±0.10 ^{a}	35.3±0.20 ^{f}	26.7±0.20 ^{b}
	200	44.5±0.04 ^{i}	36.3±0.15 ^{h}	32.3±0.10 ^{h}
	400	38.6±0.15 ^{bc}	38.3±0.20 ^{j}	34.6±0.05 ^{i}
Bitter kola	100	39.5±0.11 ^{d}	33.8±0.05 ^{c}	25.3±0.05 ^{a}
	200	36.6±0.30 ^{a}	33.2±0.05 ^{b}	24.9±0.10 ^{a}
	400	39.7±0.00 ^{d}	35.7±0.05 ^{fg}	26.5±0.10 ^{b}
Lemon grass	100	38.9±0.15 ^{c}	34.8±0.15 ^{e}	27.3±0.05 ^{c}
	200	38.3±0.20 ^{b}	36.3±0.05 ^{h}	28.6±0.10 ^{d}
	400	40.8±0.05 ^{e}	34.7±0.20 ^{e}	30.4±0.10 ^{f}
Positive control (Arthemether + Lumafantrine)	5	43.3±0.10 ^{h}	39.6±0.15 ^{k}	47.4±0.15 ^{j}
Negative control		47.3±0.20 ^{j}	32.7±0.20 ^{a}	29.7±0.15 ^{e}

Mean± Standard error. Similar values in the same row that have the same superscript are not statistically different ($P<0.05$) at the 5% level.

4.1.8 Effect of combined ethanolic plant extracts on the packed cell volumes (PCV) of *P. berghei*-infected mice

The pre-infection PCV of the negative control group was 37.0% on the 1st day of treatment and at 7th day post infection it decreased to 29.7% as compared to the positive control group (42.8– 48.5%) and the treated groups. The groups treated with the combined ethanolic extracts of bitter leaf and walnut (34.0– 38.3%); and bitter leaf and lemon grass (36.3– 41.7%) caused a significant increase in the PCV as compared to the negative control group.

Table 4.8: Combined Effect of Ethanolic Plant Extracts on the Packed Cell Volumes (PCV) of *P. berghei*-Infected Mice.

Crude Extracts	Dose (mg/kgbw)	PCV(%)		
		Before Infection	Treatment at Day 1	Treatment at Day 7
Bitter leaf + Bitter kola	400	37.7±0.02 [b]	34.7±0.02 [b]	35.7±0.02 [b]
Walnut + Bitter leaf	400	36.3±0.02 [b]	34.0±0.02 [b]	38.3±0.02 [b]
Bitter leaf + Lemon grass	400	40.0±0.02 [b]	36.3±0.02 [b]	41.7±0.02 [b]
Positive control (Arthemether + Lumafantrine)	5	38.7±0.02 [b]	42.8±0.02 [b]	48.5±0.02 [b]
Negative control		40.3±0.02 [b]	37.0±0.02 [b]	29.7±0.02 [b]

Mean± Standard error. Similar values in the same row that have the same superscript are not statistically different (P<0.05) at the 5% level.

4.1.9 Curative activity of aqueous and ethanolic extracts in *P. berghei*-infected mice

Figure 4.1 to 4.12 shows the respective curative activity of aqueous and ethanolic extracts in *P. berghei* -Infected Mice. The parasite density in all the groups treated with aqueous and ethanolic plant extarcts had its peak at day 4. There was reduction in parasite density from day 5 until the end of the treatment. At day 2, the parasite density of the group treated with standard drug (Arthemether + Lumafantrine) started reducing unlike the group that was not treated (Negative control group), whose parasite density was increasing.

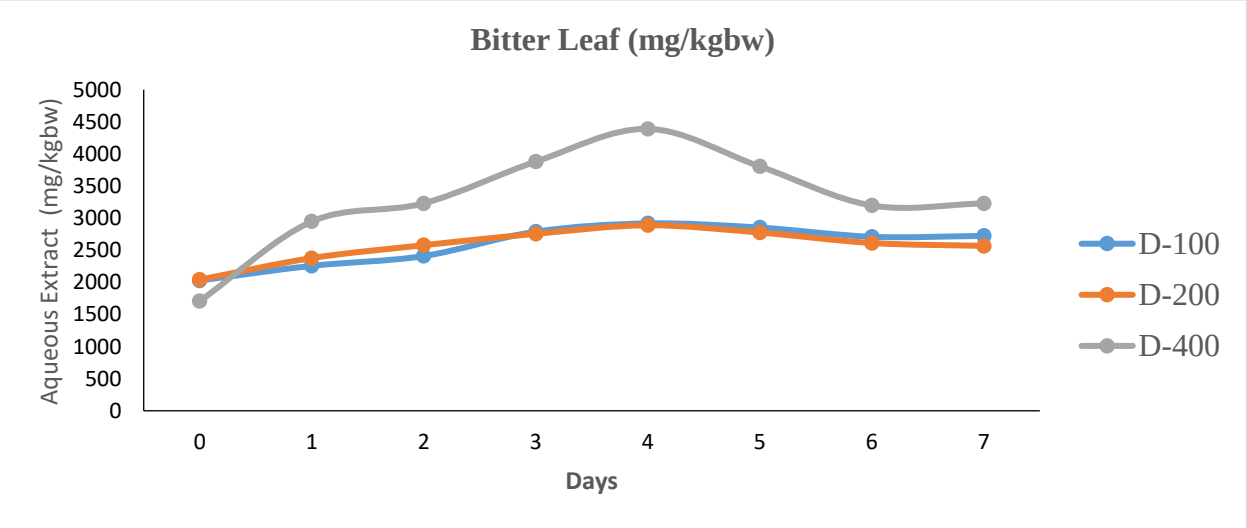


Figure 4.1: Curative Activity of Aqueous Extract of *Vernonia amygdalina* (Bitter leaf) in *P. berghei* -Infected Mice

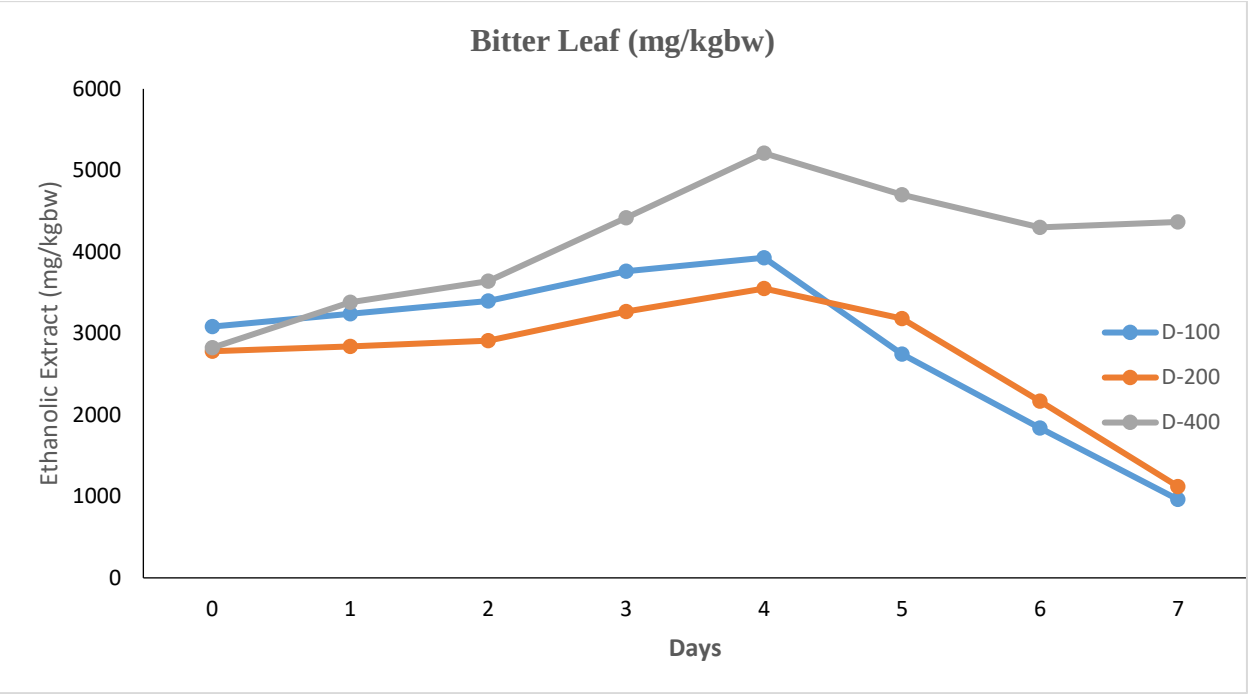


Figure 4.2: Curative Activity of Ethanolic Extract of *Vernonia amygdalina* (Bitter leaf) in *P. berghei* Infected Mice

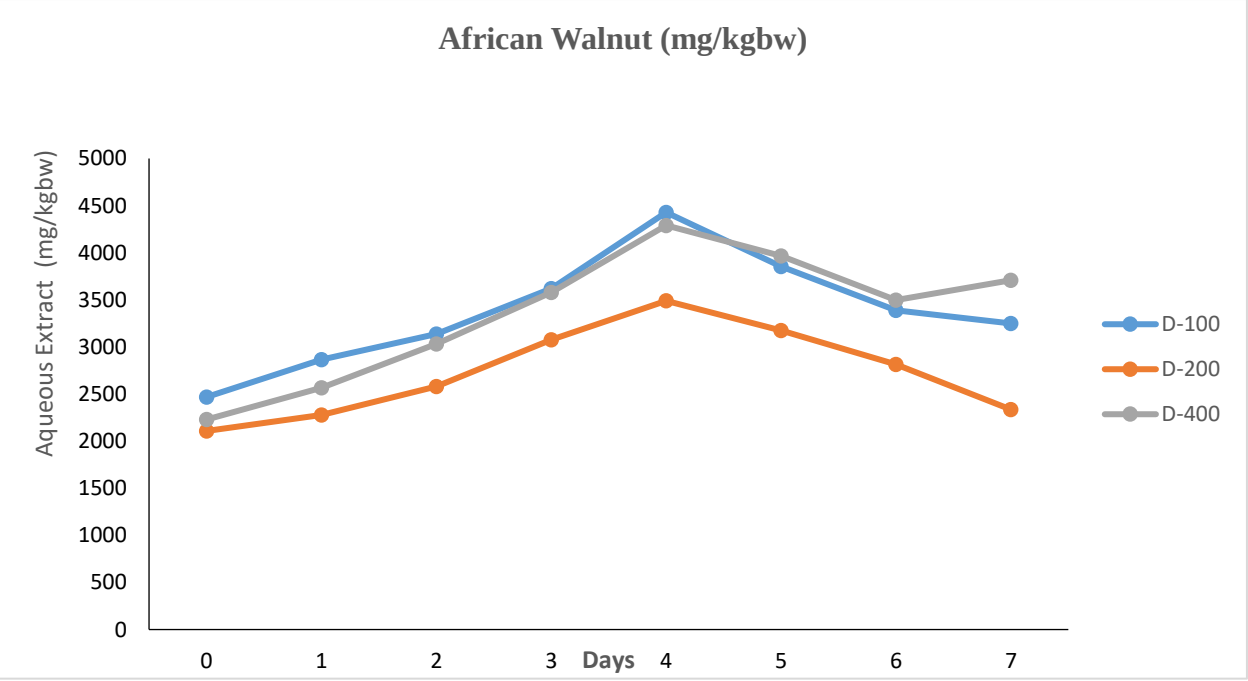


Figure 4.3: Curative Activity of Aqueous Extract of *Tetracarpidium conophorum (African Walnut)* in *P. berghei*-Infected Mice

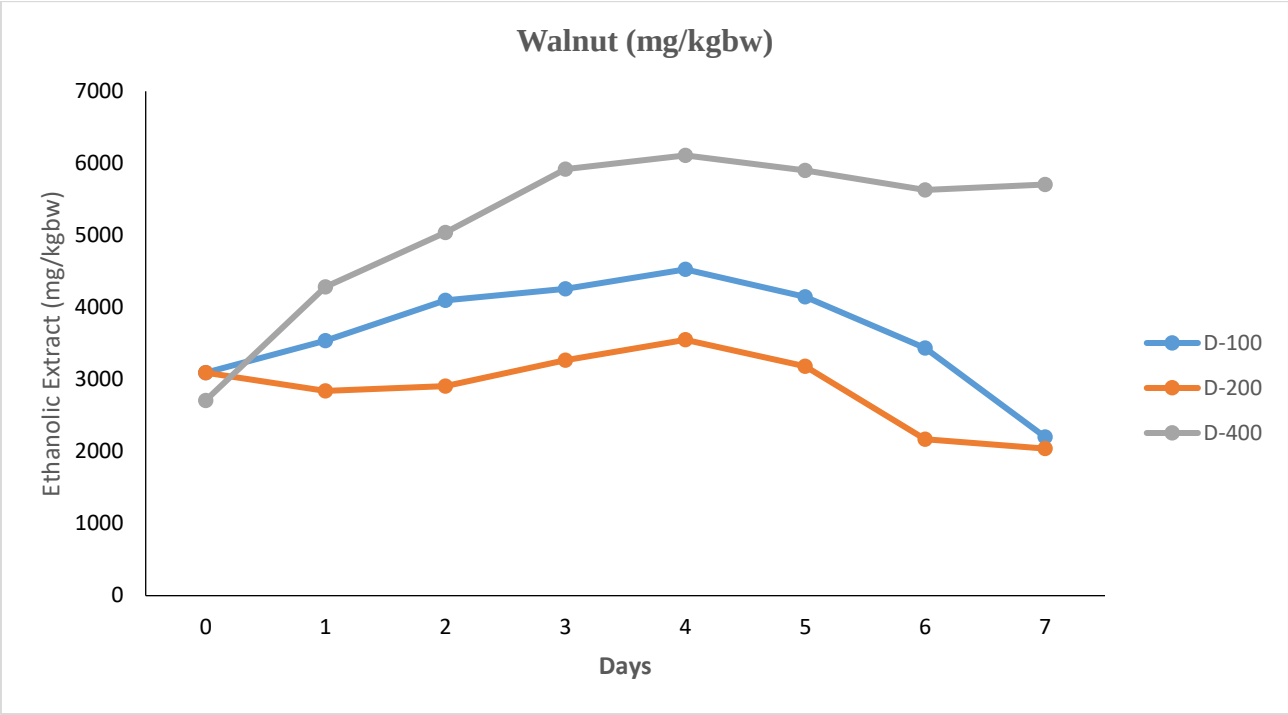


Figure 4.4: Curative Activity of Ethanolic Extract of *Tetracarpidium conophorum (African Walnut)* in *P. berghei*-Infected Mice

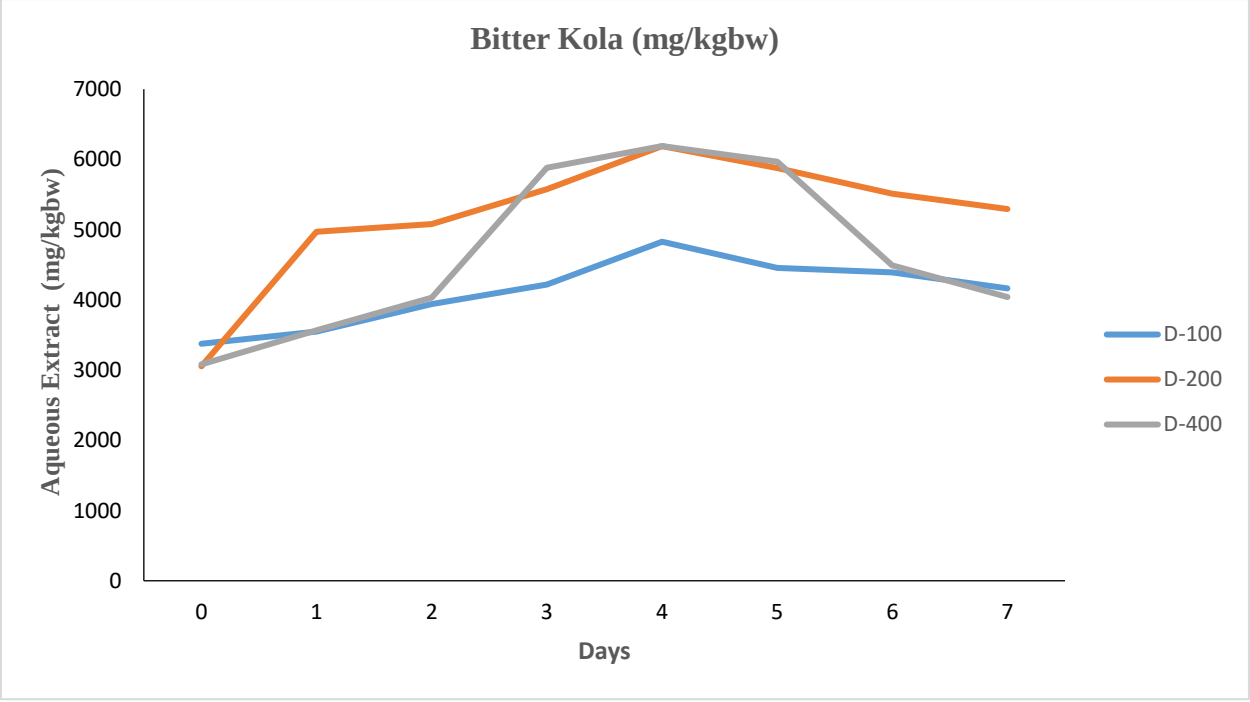


Figure 4.5: Curative Activity of Aqueous Extract of *Garcinia kola (Bitter kola)* in *P. berghei*-Infected Mice

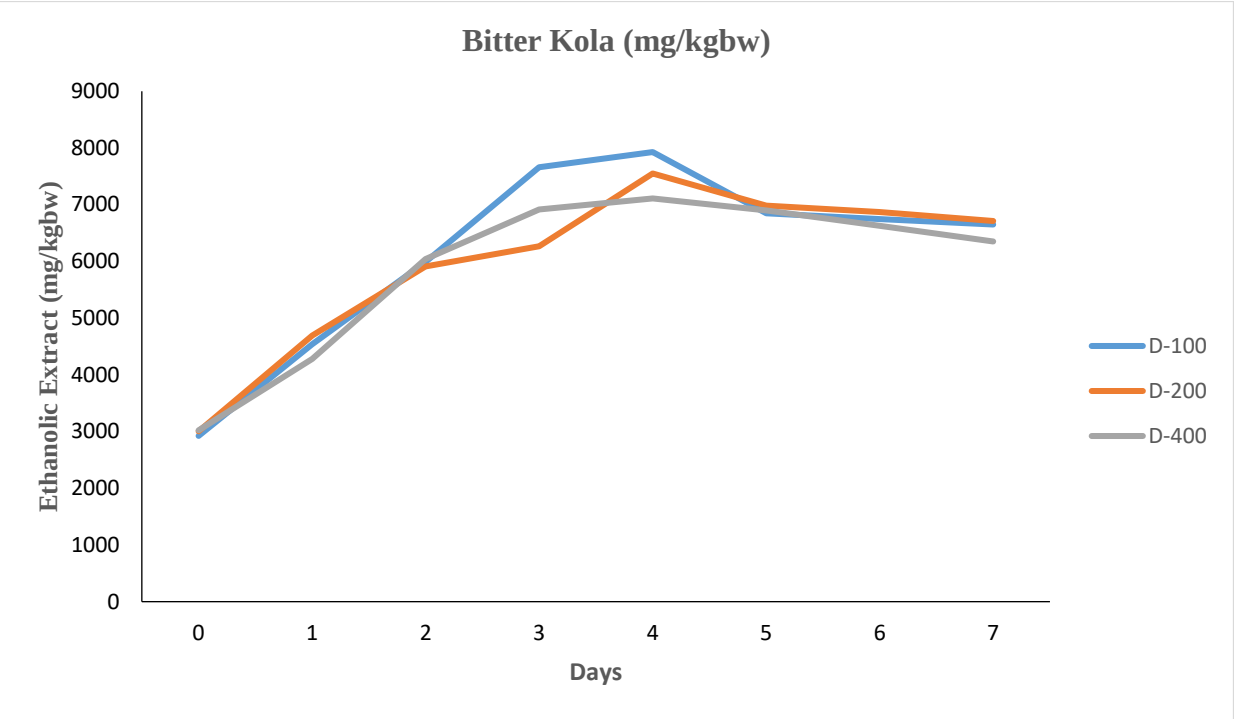


Figure 4.6: Curative Activity of Ethanolic Extract of *Garcinia kola (Bitter kola)* in *P. berghei*-Infected Mice

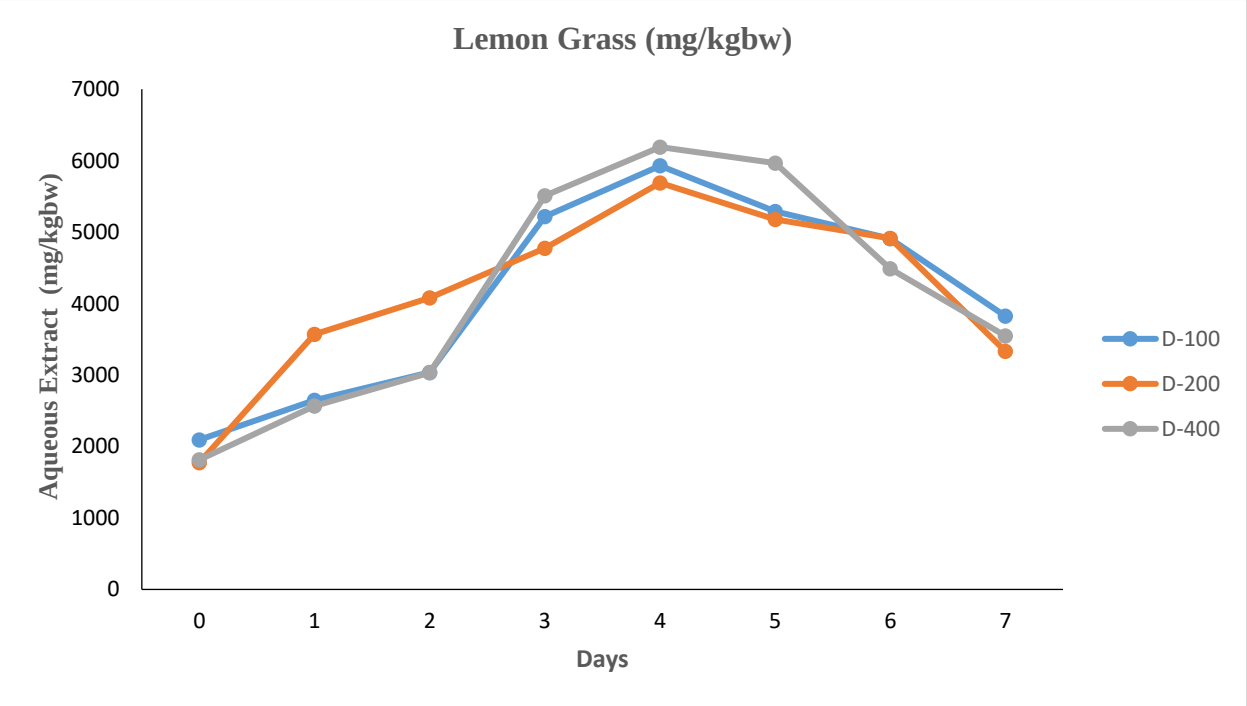


Figure 4.7: Curative Activity of Aqueous Extract of *Cymbopogon citratus (*Lemon grass*)* in *P. berghei* Infected Mice

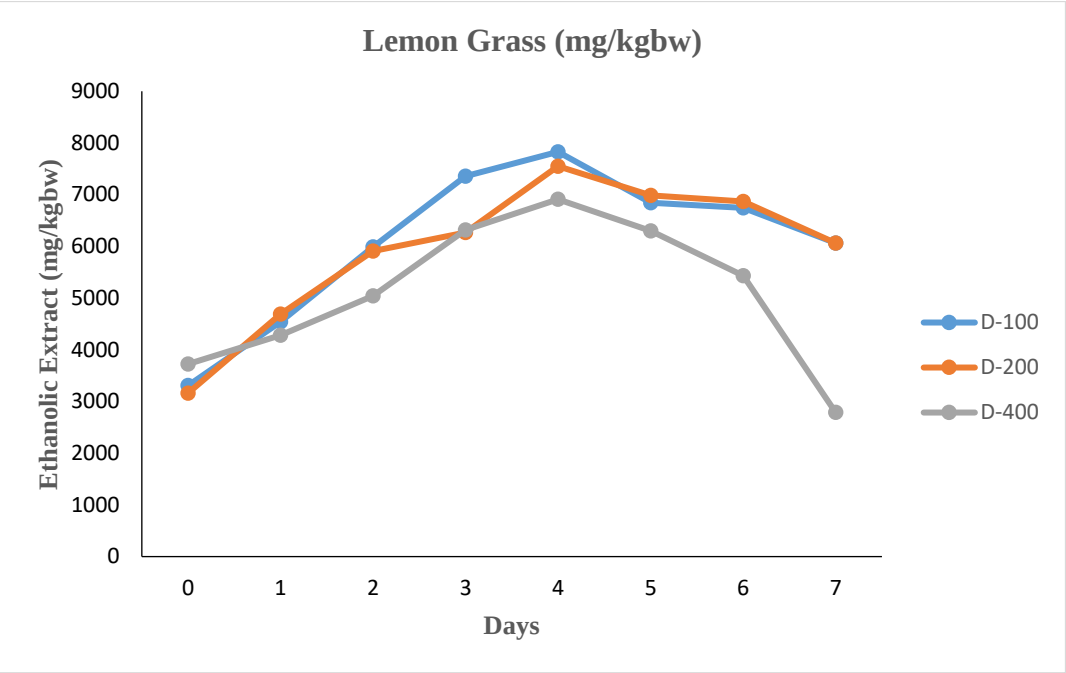


Figure 4.8: Curative Activity of Ethanolic Extract of *Cymbopogon citratus (*Lemon grass*)* in *P. berghei*-Infected Mice

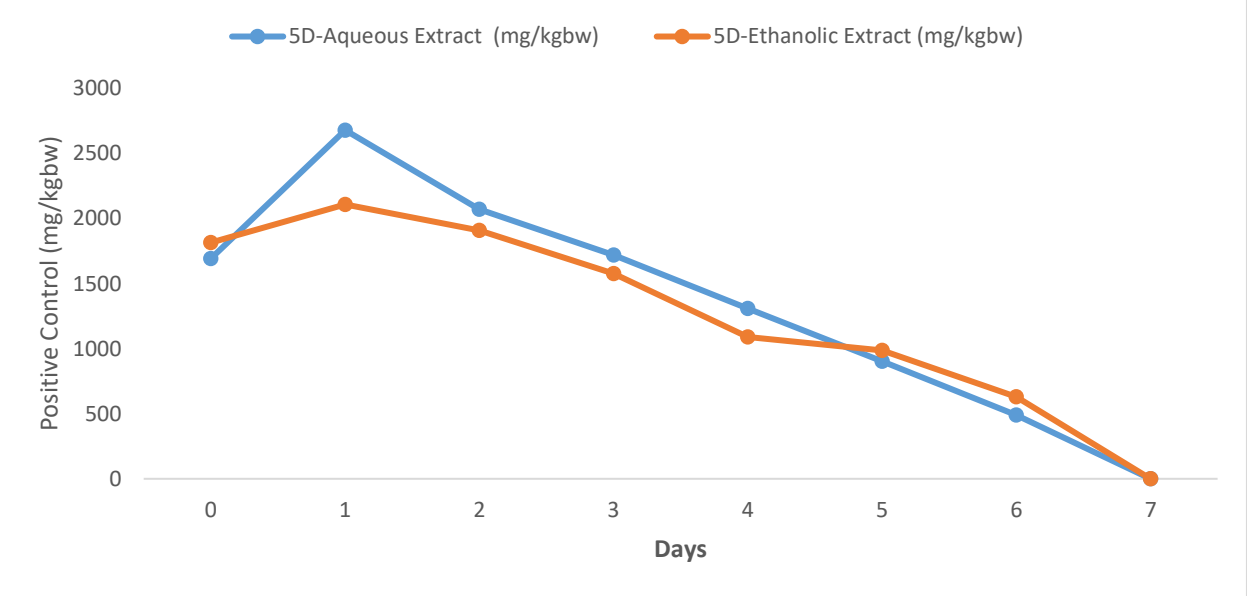


Figure 4.9: Curative Activity of standard drug (Arthemether+Lumafantrine) in *P. berghei*-Infected Mice

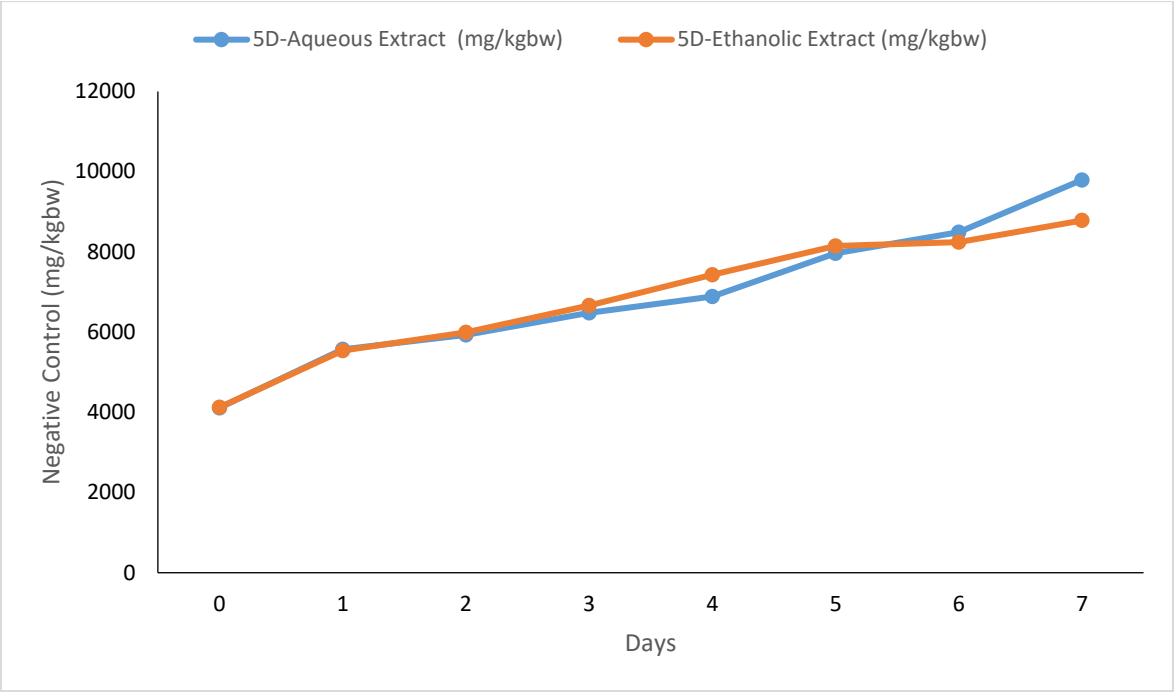


Figure 4.10: Parasite density in *P. berghei* Infected Mice (Negative control)

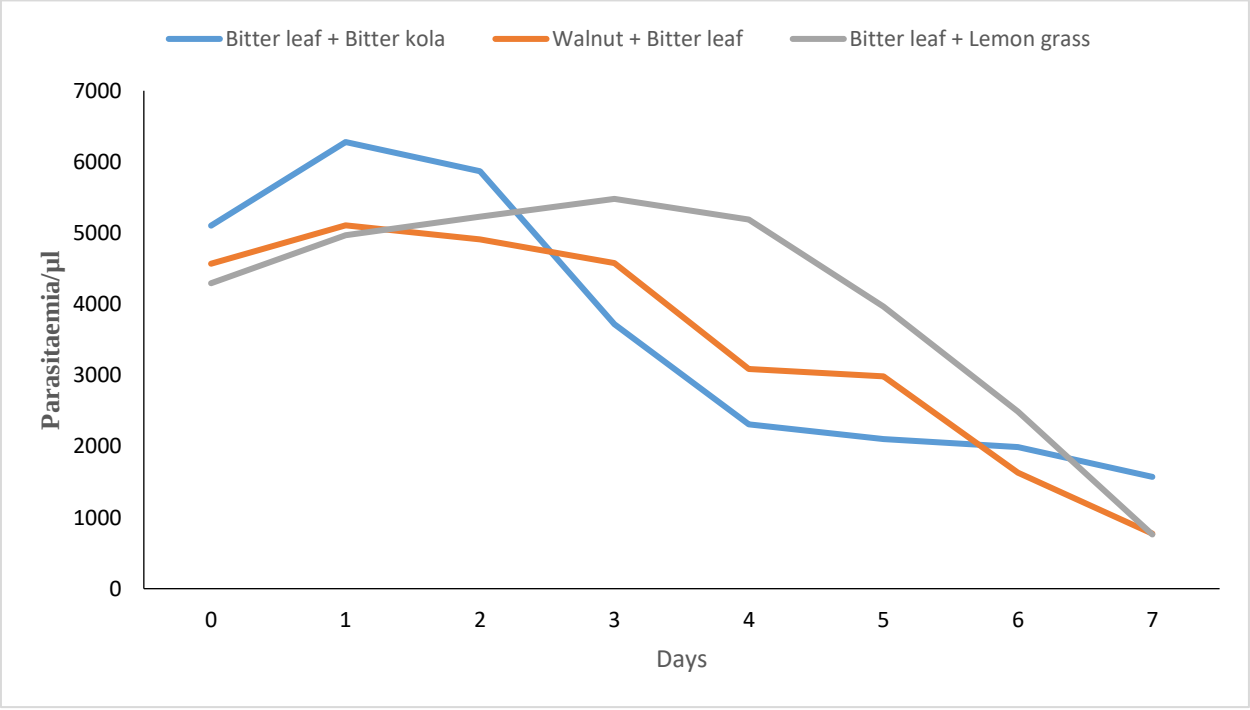


Figure 4.11: Curative Activity of Combined Extracts in *P. berghei* Infected Mice

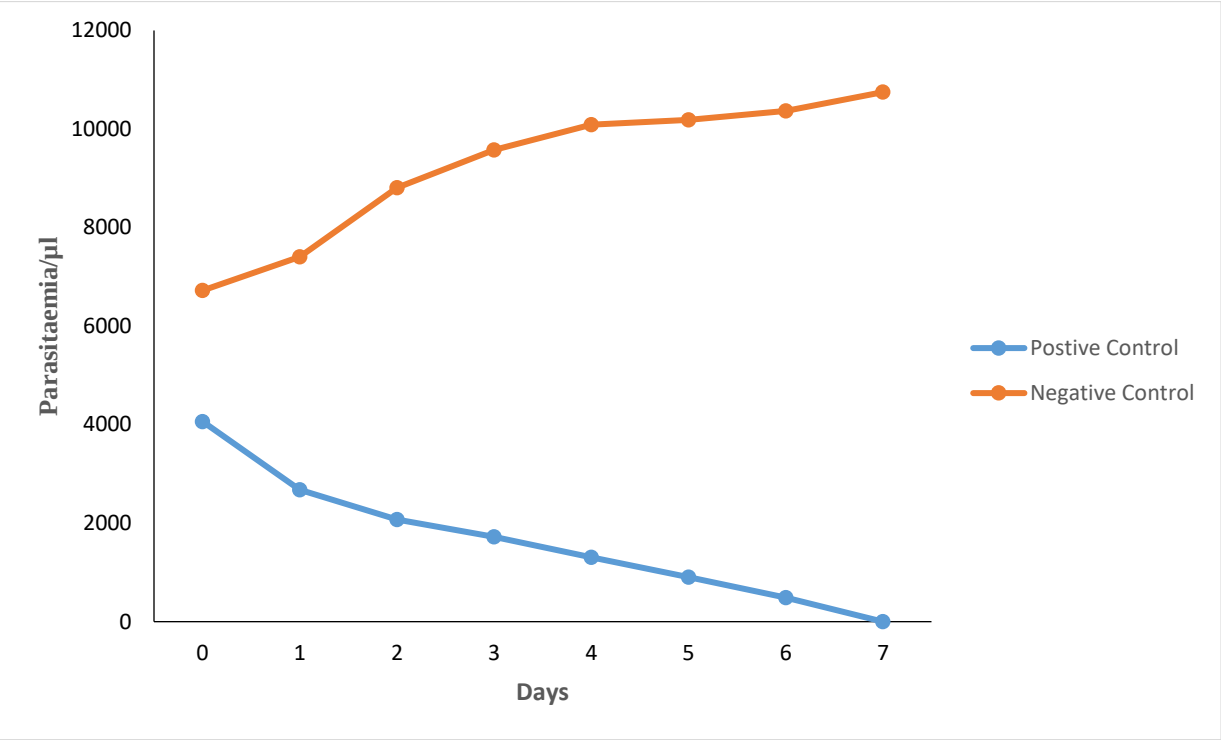


Figure 4.12: Curative Activity obtained in positive and negative control

4.1.10 Percentage clearance activity of the plant extracts in *P. berghei*-infected mice

The results of the evaluation of the percentage of *P. berghei* infection that was cleared by the plant extracts in the deficient mice are shown in Table 4.10. There was no significant difference in the levels of parasitaemia between the groups of treated mice after 72 hours of inoculating with *P. berghei* (day 0). However, the findings showed that the percentage of parasitemia dropped considerably in the groups that were treated with aqueous and ethanolic extracts when compared to the group that served as the negative control. This was the case after treatment for a total of seven days in a row. The extract also demonstrated a modest dose-dependent increase in the proportion of *P. berghei* that was cleared. When compared to the other concentrations that were given to the different groups, the ethanolic extract of bitter leaf that was given at 100 mg/kg and 200 mg/kg generated the greatest percentage clearance of 67% and 65% respectively. This is equivalent to the conventional

antimalarial medicine, artemether and lumefantrine, which was given to the group that served as the positive control (100%). Therefore, the antimalarial impact of the ethanolic extract of bitter leaf at a dose of 100 mg/kg body weight is equivalent to that of a typical antimalarial medication.

Table 4.10: Percentage of Parasite Suppression of Aqueous and Ethanolic Plant Extracts on *P. berghei*-Infected Mice.

Crude Extract	Dose (mg/kgbw)	Aqueous Extract % Suppression	Ethanolic Extract % Suppression
Bitter Leaf	100	54	67
	200	55	65
	400	58	39
Walnut	100	48	50
	200	52	45
	400	49	37
Bitter kola	100	36	28
	200	29	22
	400	35	30
Lemon grass	100	48	30
	200	52	30
	400	55	40
Positive control (Arthemether+ Lumafantrine)	5	100	100
Negative control		00	00

4.1.11 Percentage clearance activity of combined plant extracts in *P. berghei*-infected mice

Table 4.11 shows the assessment of percentage clearance activity of the combined plant extracts on *P. berghei* in the infected mice. After treatment for 7 consecutive days, the results showed that the percentage parasitemia decreased significantly ($p<0.05$) in the groups treated with the combined extracts. Combined extracts of bitter leaf and lemon grass recorded the highest percentage clearance (72%), followed by combined extracts of bitter leaf and walnut (71%) and the least was the combined extracts of bitter leaf + bitter kola (63%). The % clearance effect of the combined extracts of bitter leaf and lemon grass is comparable with that of the standard antimalarial drug (100%).

Table 4.11: Percentage of Parasite Suppression of Combined Plant Extracts on *P. berghei*-Infected Mice.

Crude Extracts	Dose (mg/kgbw)	Parasitaemia/µl		
		Day 0	Day 7	%Suppression
Bitter leaf + Bitter Kola	400	5104±2.00 [d]	1572±0.50 [d]	63
Walnut + Bitter leaf	400	4567±3.50 [c]	774±1.00 [c]	71
Bitter leaf + Lemon grass	400	4296±3.50 [b]	762±1.50 [b]	72
Positive control (Arthemether+ Lumafantrine)	5	4063±2.50 [a]	0.00±0.00 [a]	100
Negative control	--	6722±2.00 [e]	10748±1.50 [e]	-

Mean± Standard error. Similar values in the same row that have the same superscript are not statistically different ($P<0.05$) at the 5% level.

4.1.12 Mean survival time of experimental animals infected with *P. berghei* and treated with aqueous and ethanolic plant extracts

The survival times of infected mice treated with aqueous and ethanolic extracts were significantly different from the survival times of animals treated with the conventional treatment when compared to the comparative mean survival time (MST) (Table 4.12). The mean survival time of mice that had been infected with *P. berghei* and treated with aqueous extracts of bitter leaf and walnut, as well as the standard medication, was substantially longer than the MST of mice that had not been treated at all. However, after 12 days of therapy, there was no mortality observed in the groups that were given aqueous extracts of bitter leaf, walnut, lemon grass, and the conventional medicine after infection with *P. berghei*.

Table 4.12: Mean Survival Time of Experimental Mice Infected with *P. berghei* and Treated with Aqueous and Ethanolic Plant Extracts.

		Mean survival time	
Crude extracts	**Groups**	**Aqueous extract**	**Ethanolic extract**
		Number of Days	**Number of Days**
Bitter leaf	C	14	20
	D	19	19
	E	17	10
Walnut	C	26	19
	D	29	18
	E	11	10
Bitter kola	C	14	11
	D	07	09
	E	16	08
Lemon grass	C	13	10
	D	15	09
	E	21	16
Positive control	A	>30	>30
Negative control	B	07	7

4.1.13 Hematological analysis of the effect of aqueous plant extracts on *P. berghei*-infected mice

Table 4.13 presents the results of a haematological examination, which revealed dose-dependent variations in packed cell volume (PCV), haemoglobin (HB) levels, red blood cell (RBC), white blood cell (WBC), and platelet (PLC) counts across all groups. The PCV, HB, RBC, and WBC values of mice treated with bitter kola were the lowest, whereas the PLC value of mice treated with bitter leaf were the lowest. Mice in the positive control group had the greatest PCV, HB, RBC, and PLC levels. Treatment with lemon grass extract resulted in a dose-dependent increase in the mean corpuscular volume (MCV) of mice compared to controls. White blood cell (WBC) counts were considerably lower in all groups except the walnut extract group, with the greatest WBC numbers seen in the bitter kola group. There was no statistically significant change in the mean Lymphocytes count in untreated and treated mice. Differences in eosinophil and basophil count were statistically significant across all groups of mice. The neutrophil count in all the mouse groups decreased, and there was a statistically significant difference between the neutrophil counts of the different groups.

Table 4.13: Haematological Analysis of *P. berghei*-Infected Mice Treated with Aqueous Plant Extracts

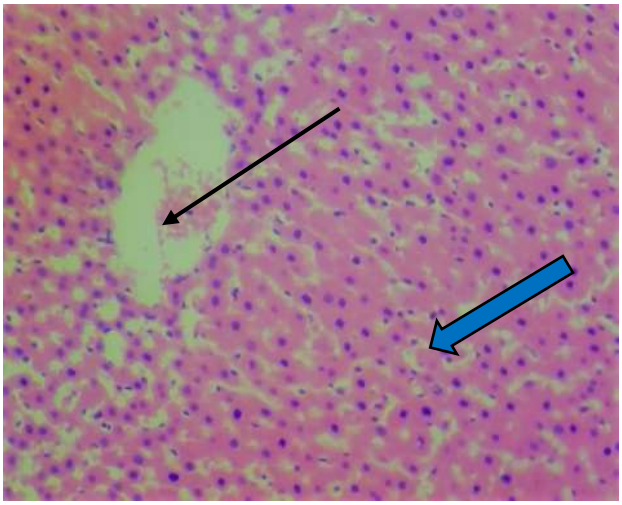
Crude extract	Groups	PCV (%)	MCV (fi)	MCH (pg)	MCHC (g/dL)	RBC (10^{12}/L)	PLC	TWBC (10^{6}/L)
Bitter leaf	C	16.8±0.31^{c}	35.33±0.25^{d}	45.27±2.11^{d}	19.10±1.23^{a}	39.25±3.45^{ab}	7.20±1.23^{b}	129.32±4.25^{ab}
	D	18.40±1.11^{c}	39.25±0.98^{d}	34.49±2.11^{b}	23.11±1.15^{b}	36.86±3.33^{a}	8.41±1.25^{b}	120.30±5.25^{a}
	E	13.70±2.50^{b}	30.12±3.33^{c}	29.19±0.99^{b}	16.47±2.82^{a}	30.82±2.19^{a}	9.10±0.58^{b}	108.29±10.24^{a}
Walnut	C	17.25±2.11^{c}	38.28±2.62^{d}	46.13±1.19^{d}	20.29±1.81^{ab}	39.39±2.49^{ab}	8.33±0.52^{b}	127.21±8.96^{ab}
	D	20.13±1.18^{c}	42.86±2.99^{e}	49.14±4.26^{d}	26.91±1.87^{b}	45.23±3.12^{b}	8.73±1.95^{b}	149.32±9.58^{c}
	E	13.72±2.44^{b}	31.65±2.65^{c}	36.98±3.11^{c}	18.92±1.22^{a}	40.11±3.70^{ab}	7.13±0.51^{b}	145.33±7.89^{c}
Bitter kola	C	10.41±2.15^{b}	29.24±2.17^{c}	30.45±0.29^{b}	15.56±0.67^{a}	35.49±1.32^{a}	6.20±1.19^{a}	132.19±2.22^{b}
	D	5.41±0.27^{a}	20.31±0.79^{b}	22.13±1.50^{a}	19.41±2.50^{a}	30.86±3.45^{a}	5.67±2.14^{a}	112.13±6.89^{a}
	E	6.12±0.74^{a}	25.20±1.85^{c}	24.92±1.49^{a}	19.23±2.10^{a}	32.22±3.19^{a}	5.81±0.25^{a}	121.88±5.26^{a}
Lemon grass	C	15.33±2.75^{c}	32.41±1.98^{c}	38.65±2.15^{c}	21.32±1.99^{ab}	37.61±3.19^{a}	8.10±0.79^{b}	136.45±7.21^{b}
	D	10.40±1.93	38.21±2.56^{d}	42.91±5.27^{d}	16.85±2.25^{a}	40.42±1.92^{ab}	6.50±2.22^{b}	143.21±11.23^{c}
	E	12.91±3.12^{b}	36.21±1.99^{d}	40.31±1.19^{d}	19.21±3.11^{a}	31.86±1.10^{a}	7.31±1.14^{b}	123.10±9.33^{ab}
Positive control		16.30±0.56^{c}	46.31±1.11^{e}	21.27±0.66^{a}	23.31±1.29^{b}	35.49±1.23^{a}	11.20±0.97	156.31±2.98^{c}
Negative control		3.60±1.11^{a}	15.25±0.89^{a}	37.98±1.10^{c}	25.29±0.91^{b}	38.39±2.44^{a}	3.22±0.65^{a}	116.91±8.91^{a}

Mean± Standard error. Values in the same row with same superscript are not statistically different (P<0.05) at the 5% level.

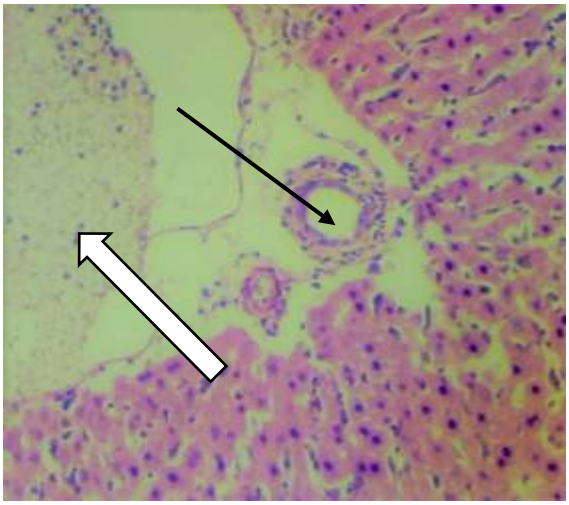
4.1.14 Histological analysis of the effect of plant extracts on the liver of *P. berghei*-infected mice

4.1.14.1 Liver section of P. berghei- infected mice treated with Vernonia amygdalina extract

The impact of the extracts on the histology of the liver and kidneys was evaluated, as these organs play important roles in metabolism and excretion respectively. Mice that had been infected and then given extracts of *Vernonia amygdalina* at doses of 100, 200, and 400 mg/kg body weight had their livers sectioned. The photomicrograph revealed hepatic tissue that had maintained its architecture and was made up of cords of normal hepatocytes, as well as sinusoids and normal portal veins. Others of the hepatocytes revealed foamy cytoplasms (blue arrow), while some showed encapsulated parasites. There are no symptoms of acute or chronic damage (black arrow). No infiltration of the sinusoids was seen (shown by the white arrow), and there was no sign of any clinical lesions (Plate 1).



A



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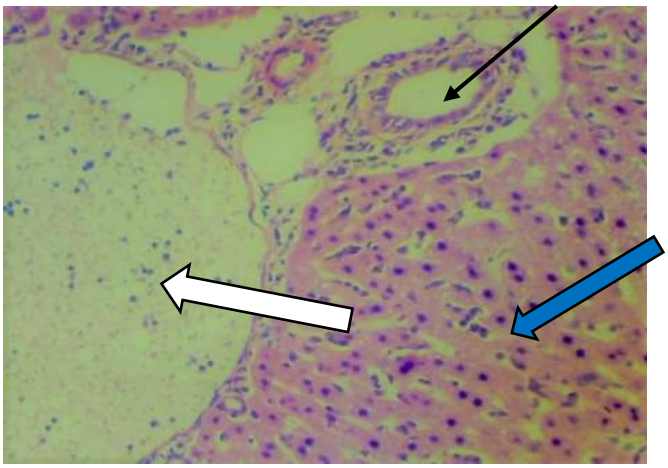


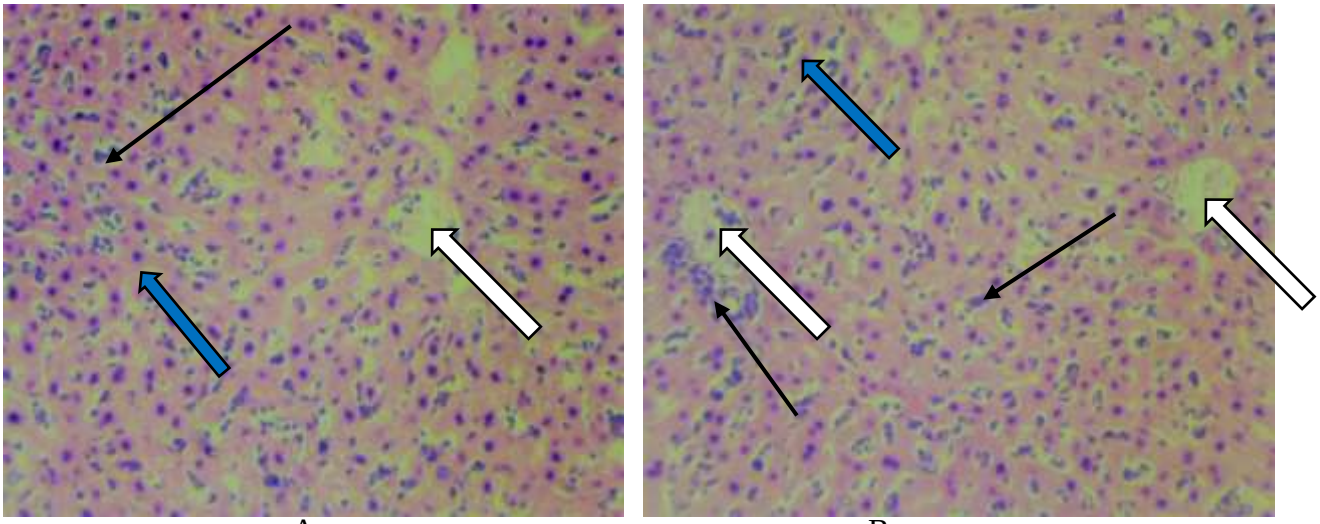
C

Plate 1: Photomicrograph of a liver section of *P. berghei*- infected mice treated with *Vernonia amygdalina* extract (100, 200 and 400 mg/kgbw respectively)

A: 100 mg/kgbw; B: 200 mg/kgbw; C: 400 mg/kgbw
Blue arrow: Hepatocytes
White arrow: Sinusoids
Black arrow: Portal vein

4.1.14.2 Liver section of infected mice treated with Garcinia kola extracts

The photomicrograph of the segment of liver seen under the microscope revealed extremely poor architecture. There was severe portal triaditis, and the portal tracts demonstrates periportal infiltration of inflammatory cells along with mild congestion of the portal vein (white arrow). Additionally, some of the hepatocytes displayed foamy cytoplasms, and some displayed cellular debris that was being consumed by Kupffer cells (blue arrow). There were significant infiltrates of inflammatory cells and just a little bit of vascular congestion seen. Kupffer cells had parasites inside of them, and the malaria pigment, haemozoin, could be seen inside of them as well (black arrow) (Plate 2).



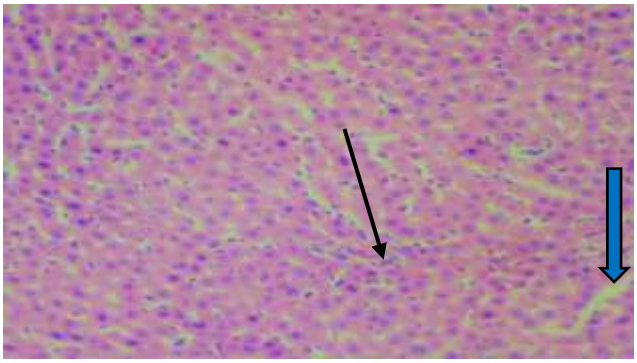
A B

Plate 2: Photomicrograph of a liver section of *P. berghei*- infected mice treated with *Garcinia kola* extracts (100 and 400 mg/kgbw)

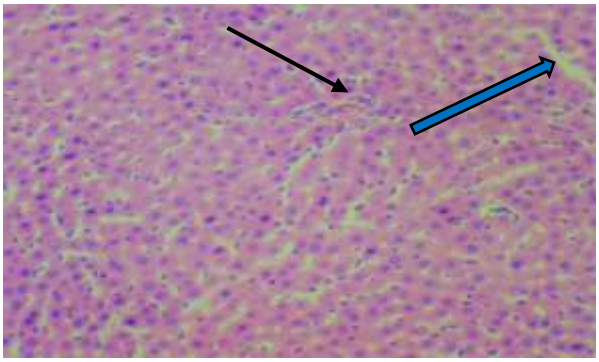
A: 100 mg/kgbw; B: 400 mg/kgbw
Blue arrow: Hepatocytes
White arrow: Portal vein
Black arrow: Sinusoids

4.1.14.3 Liver section of infected mice treated with Tetracarpidium conophorum extracts

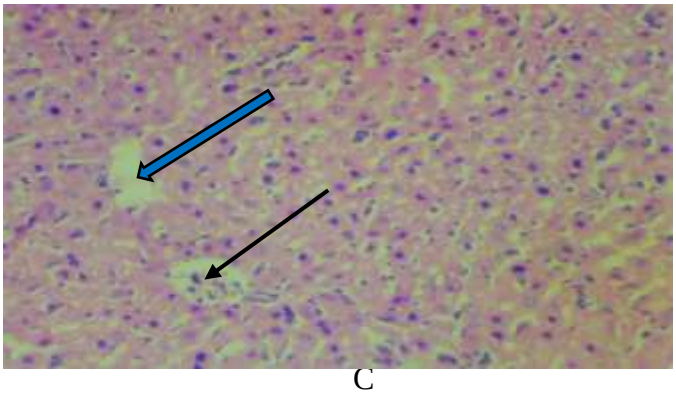
The photomicrograph of the tissue samples demonstrates hepatocytes tissue with restored architecture constituted of cords of regular hepatocytes, normal portal tracts, and a central vein with no functionalities of acute or chronic damage. However, the sinusoids were mildly packed with parasite-laden Kupffer cells that had engulfed haemozoin pigments (black arrow). Mild inflammatory cellular infiltrates were also evidenced to be prevalent around the portal zone of the liver. The morphology of the hepatocytes reveals vacuolated and spongy-like cytoplasms (blue arrow), as well as parasites that have been ingested by the cells (Plate 3).



A



B



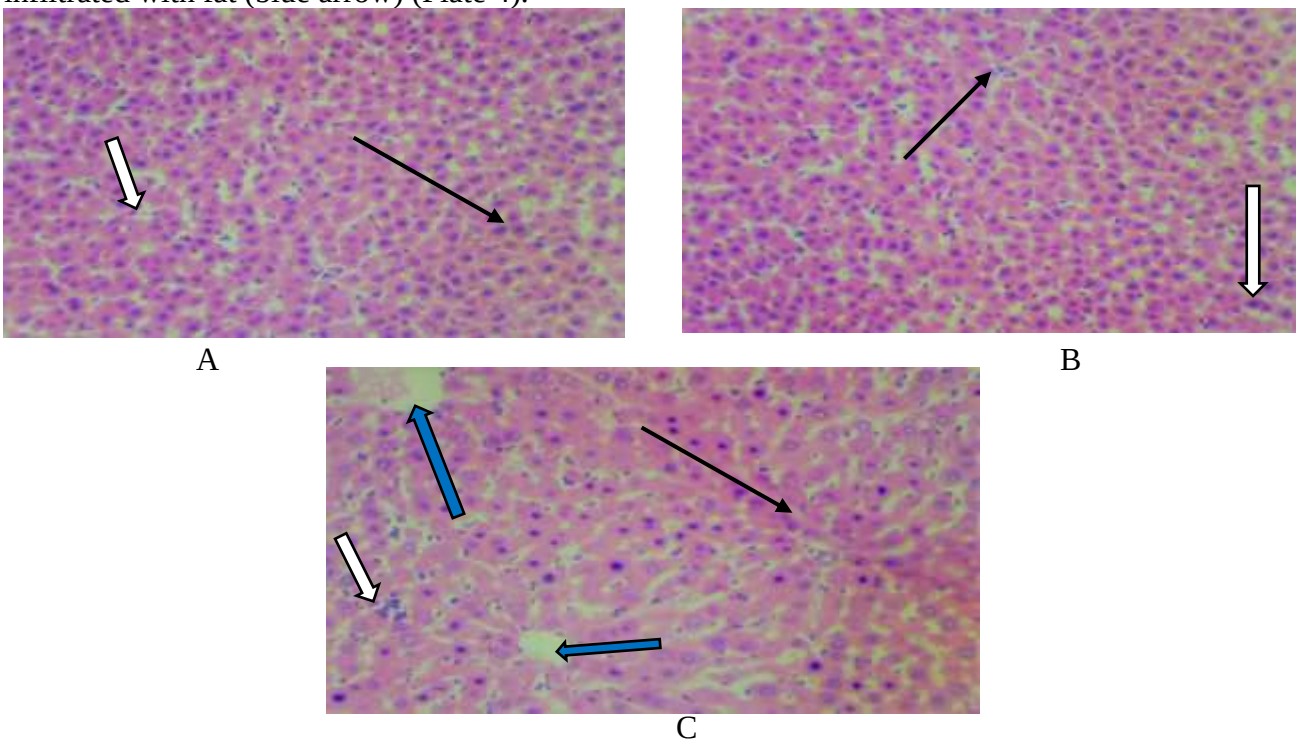
C

Plate 3: Photomicrogragh of a liver section of *P. berghei*- infected mice treated with *Tetracarpidium conophorum* extracts (100, 200 and 400 mg/kgbw respectively)

A: 100 mg/kgbw; B: 200 mg/kgbw; C: 400 mg/kgbw
Blue arrow: Hepatocytes
Black arrow: Sinusoids

4.1.14.4 Liver section of infected mice treated with Cymbopogon citratus extracts

In a dose-dependent way, there was a reduction in the amount of inflammatory cellular infiltrates seen in the liver, suggesting that the malaria infection was clearing up. On the other hand, the sinusoids had a moderate amount of Kupffer cells that were loaded with parasites and had consumed haemozoin pigment and cellular detritus (white arrow). Within the portal vein, there was only little congestion, but there was substantial infiltration of inflammatory cells around the periportal area (black arrow). Microvesicular steatosis was seen in the morphology of the hepatocytes, and the cytoplasms were vacuolated and infiltrated with fat (blue arrow) (Plate 4).



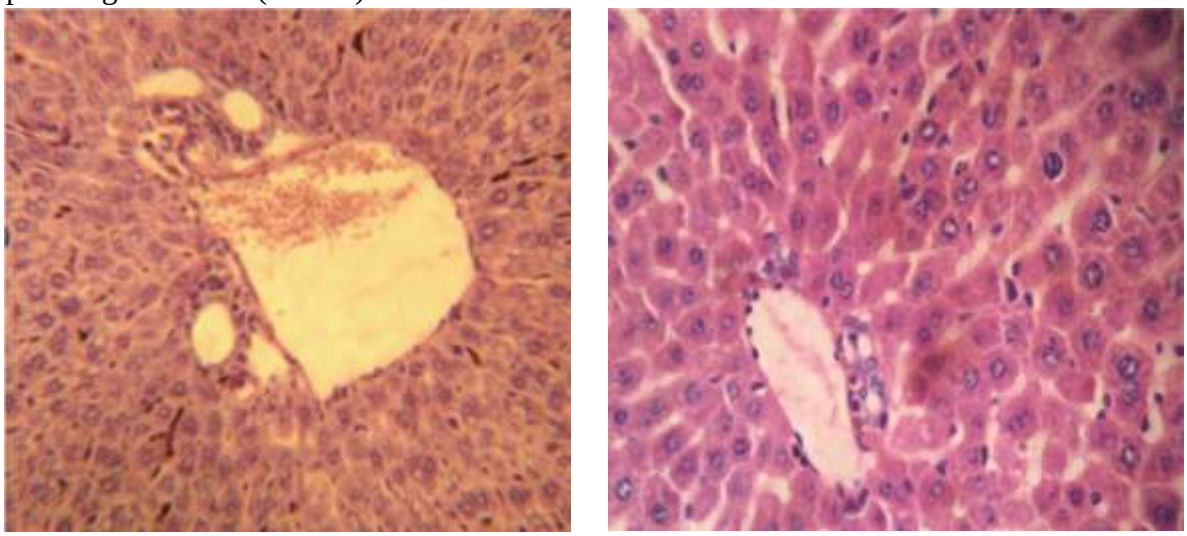
A B C

Plate 4: Photomicrogragh of a liver section of *P. berghei*- infected mice treated with *Cymbopogon citratus* extracts (100, 200 and 400 mg/kgbw)

A: 100 mg/kgbw; B: 200 mg/kgbw; C: 400 mg/kgbw
Blue arrow: Hepatocytes
White arrow: Sinusoids
Black arrow: Portal vein

4.1.14.5 Liver section of infected mice treated with standard drug (Arthemether + Lumafantrine) and uninfected mice

The histological characteristics of the liver sections were normal, and they were constituted of hepatocytes, portal veins, and sinusoids. The morphology of the hepatocytes looked to be normal, and there was no infiltration of the sinusoids. There was also no evidence of any pathological lesion (Plate 5)



A B

Plate 5: Photomicrogragh of a liver section of *P. berghei*- infected mice treated with Arthemether+Lumafantrine and uninfected mice

A: Mice treated with Arthemether+Lumafantrine
B: Uninfected mice

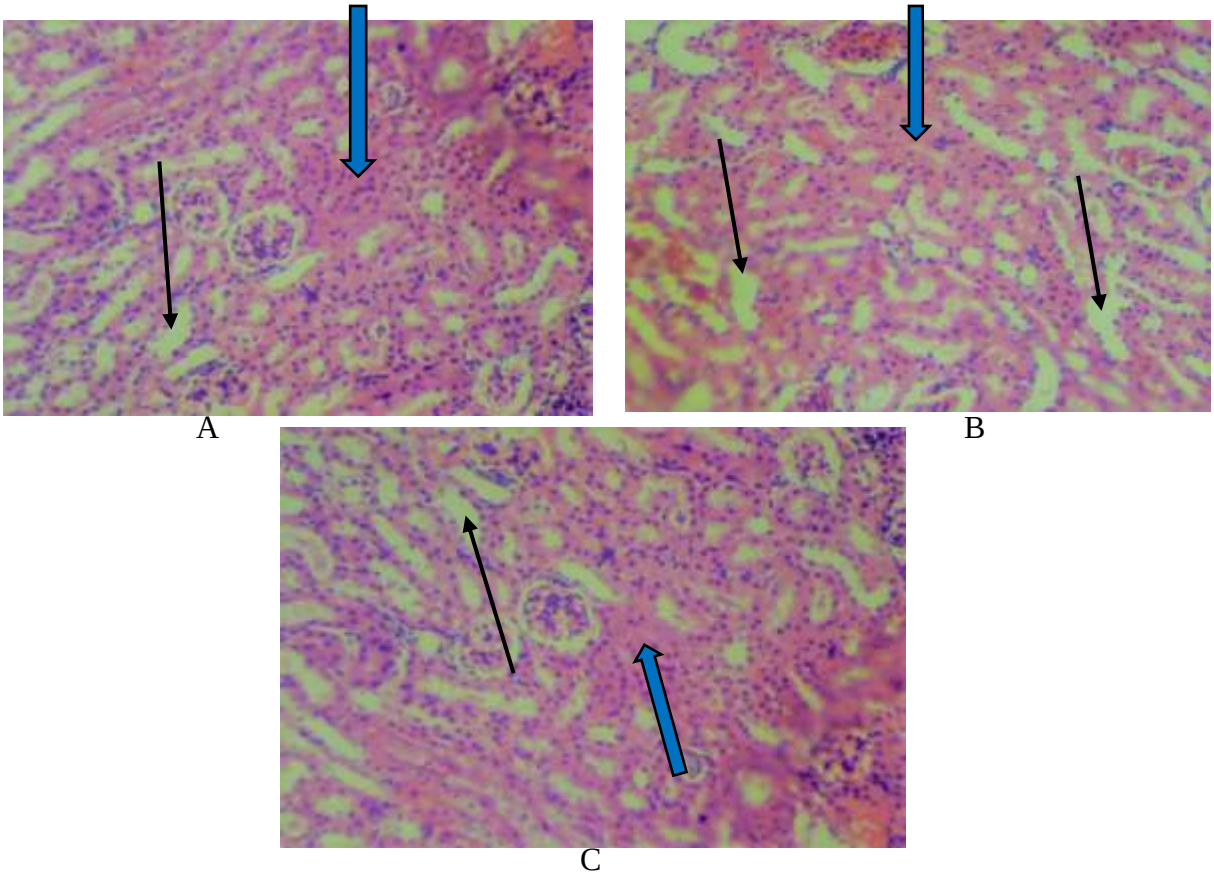
4.1.15 Histological analysis of the effect of plant extracts on the kidney of *P. berghei*-infected mice

With the administration of bitter leaf (100, 200 and 400 mg/kgbw), the renal tubule with cortex appears dilated; the group that was treated with bitter kola demonstrated that the glomeruli appear normal. However, with 400 mg/kgbw, the majority of the glomeruli were mildly obscured with mild interstitial nephritis, and the renal tissue had leukocyte infiltration, edoema exudate, and necrotic Also, the histology samples taken from the

kidneys of mice given 100, 200 and 400 mg/kgbw of walnut and lemon grass revealed dilated but normal tubules constituted of normal glomeruli and interstitium, and there were no signs of acute or chronic kidney injury.

4.1.15.1 Kidney section of P. berghei- infected mice treated with Cymbopogon citratus extract

The photomicrograph of the kidney section showed mild slouching of epithelium of tubules in the renal cortex (black arrow) and moderate congestion of renal interstitial blood vessels



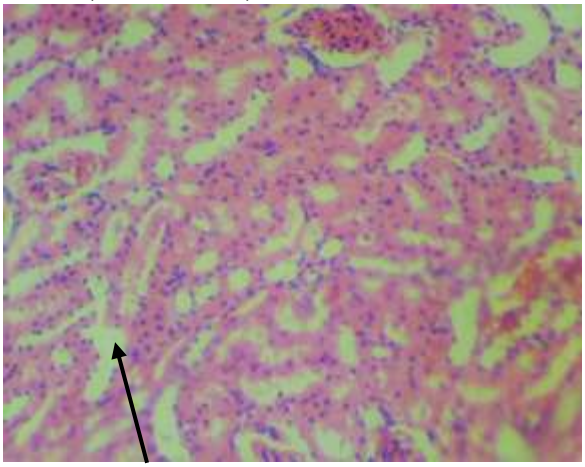
A B C

Plate 6: Photomicrogragh of a kidney section of *P. berghei*- infected mice treated with *Cymbopogon citratus* extract (100, 200 and 400 mg/kgbw).

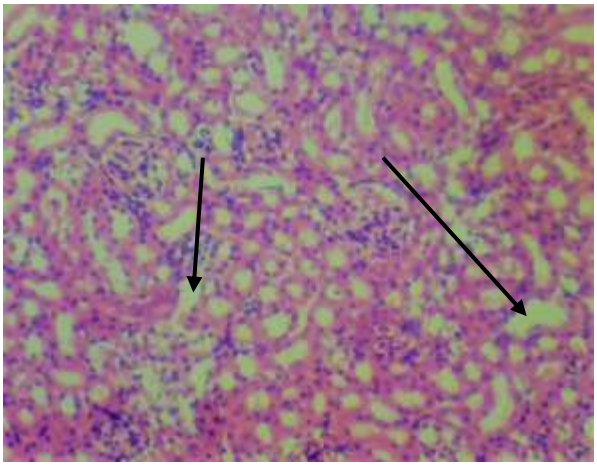
A: 100 mg/kgbw; B: 200 mg/kgbw; C: 400 mg/kgbw
Black arrow: Tubules
Blue arrow: Glomerulus

4.1.15.2 Kidney section of P. berghei- infected mice treated with Tetracarpidium conophorum extract

The slice of the kidney that was photographed for microscopy revealed renal tissue that had maintained its usual architecture, including glomeruli, tubules, and interstitial tissue. There were no outward manifestations of either acute or chronic injury. In the renal medulla, there was a considerable amount of vacuolar alteration in the epithelial cells that made up the tubules (black arrow).



A



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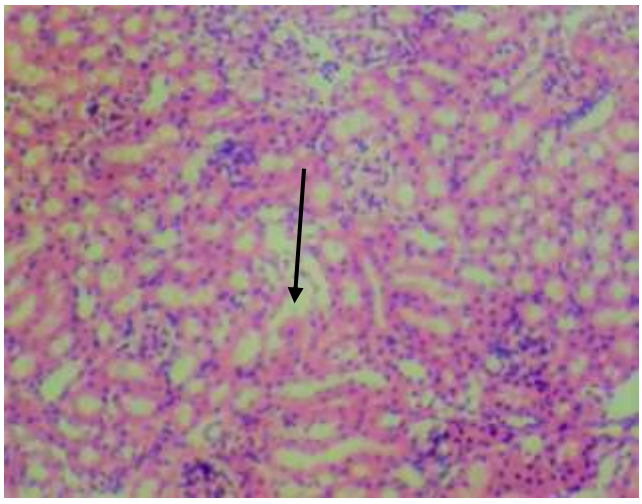


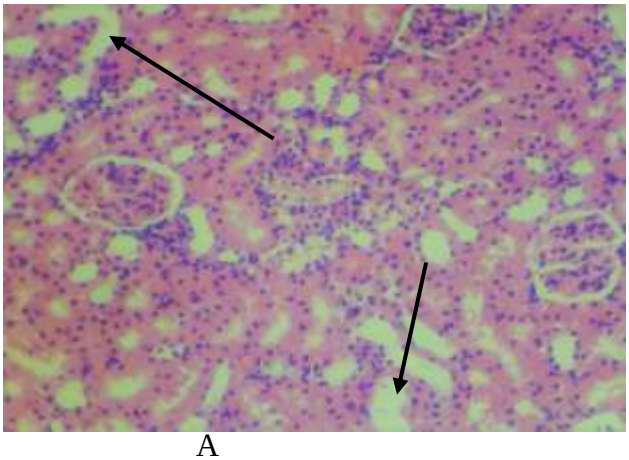
C

Plate 7: Photomicrogragh of a kidney section of *P. berghei*- infected mice treated with *Tetracarpidium conophorum* extracts (100, 200 and 400 mg/kgbw).

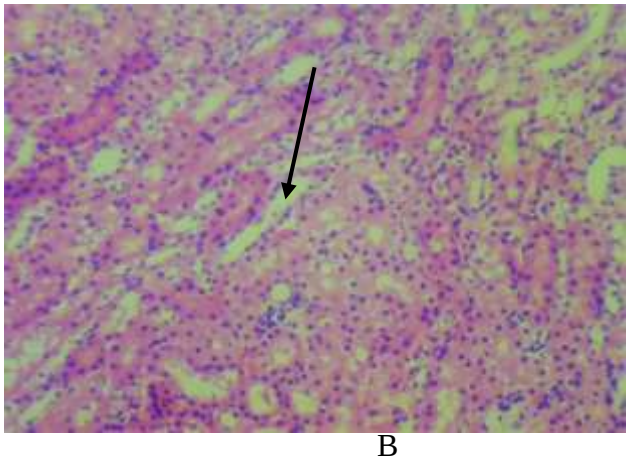
A: 100 mg/kgbw; B: 200 mg/kgbw; C: 400 mg/kgbw
Black arrow: Tubules

4.1.15.3 Kidney section of P. berghei- infected mice treated with Vernonia amygdalina extract

The slice of the kidney that was photographed for microscopy revealed renal tissue that had maintained its usual architecture, including glomeruli, tubules, and interstitial tissue. There were no indications of recent or ongoing harm found. In the renal cortex, there were just a few foci of modest slouching of the epithelial cells that lined the tubules (black arrow).



A



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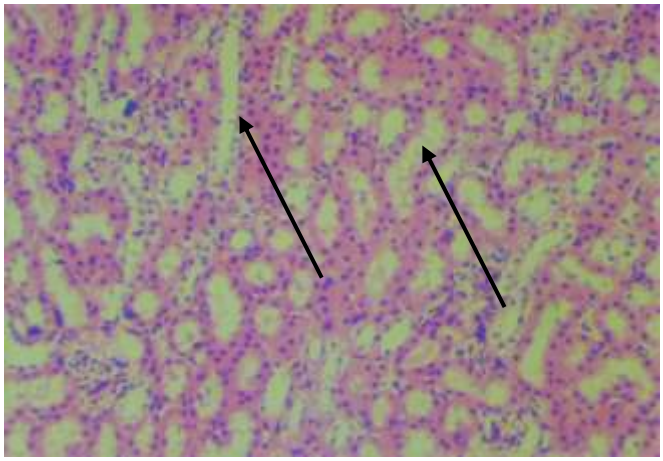


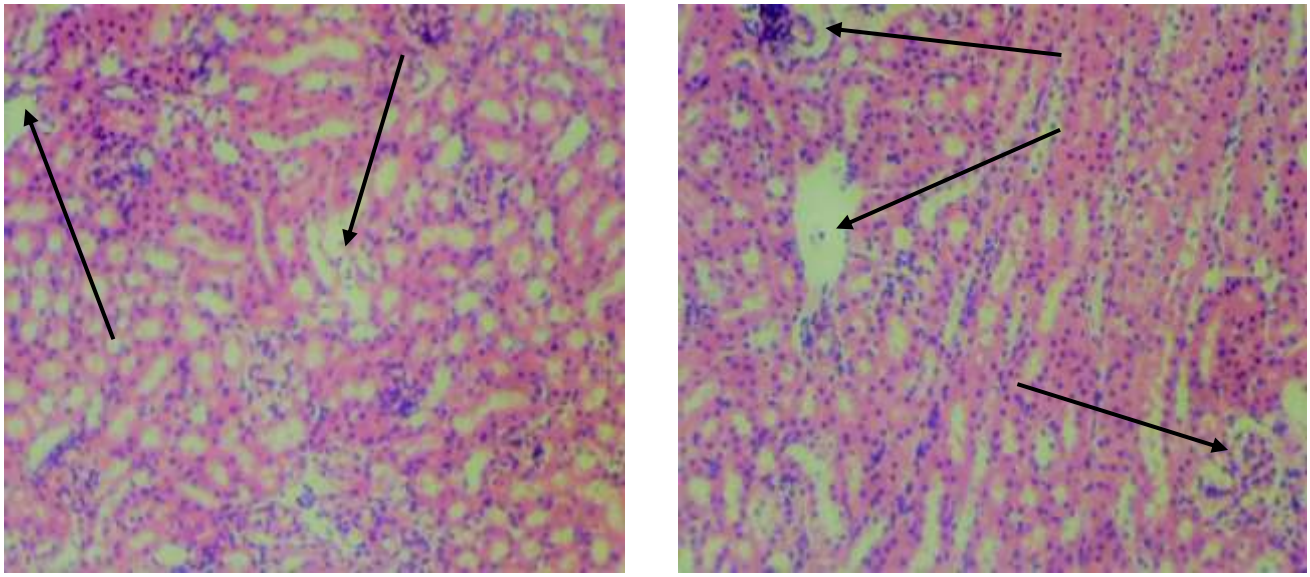
C

Plate 8: Photomicrogragh of a kidney section of *P. berghei*- infected mice treated with *Vernonia amygdalina* extracts (100, 200 and 400 mg/kgbw)

A: 100 mg/kgbw; B: 200 mg/kgbw; C: 400 mg/kgbw
Black arrow: Tubules

4.1.15.4 Kidney section of P. berghei- infected mice treated with Garcinia kola extract

The Photomicrogragh of the kidney section showed renal tissue with preserved architecture composed of normal glomeruli, tubules and interstitium. There were no features of acute or chronic damage. There was moderate vacuolar change of the epithelial cells of tubules in the renal medulla (black arrow).



A B

Plate 9: Photomicrogragh of a kidney section of *P. berghei*- infected mice treated *Garcinia kola* extract (100 and 400 mg/kgbw)

A: 100 mg/kgbw; B: 400 mg/kgbw
Black arrow: Tubules

4.2 Discussion

4.2.1 Yield of plant extracts

Variance in the extract yield is noticeable in all the plant samples and this might be due to the plant part used, the season of harvest, nature of the solvents and also the method of extraction. This research confirms previous result by Ogundolie *et al*. (2017) and Oluwatosin *et al*. (2019) that *Tetracarpidium conophorum* and *Garcinia kola* has higher yield when extracted with ethanol,

4.2.2 Phytochemical constituent of plant extracts

Phenols, flavonoids, tannins, saponins, and alkaloids, among other metabolites, were found in all of the extracts analysed by phytochemistry (Table 4.2). Antimalarial properties have been ascribed to alkaloids, saponins, and flavonoids (Ettebong *et al*., 2019). In alone or in concert, these secondary metabolites have biological effects (Shigemori *et al*., 2018). Both the aqueous and ethanolic plant extracts in this investigation include phytochemical elements with a history of medicinal usage in alternative medicine and folklore (Longdet and Adoga, 2017). This research confirms previous results by Nathan *et al*. (2016) that bitter Leaf (*Vernonia amygdalina*) extracts are effective against malaria parasites due to the presence of tannins and other limonoids. There is still lack of knowledge about the components of these plant extracts, however alkaloids like quinine are recognized to be powerful antimalarial medicines. Antiplasmodial activity may be due to the presence of alkaloids, glycosides, tannins, and flavonoids, which may work alone or in concert (Nwanjo and Alumanah, 2015). Quinine is the most well-known alkaloid used to treat malaria. The extracts' antimalarial effectiveness may also be attributable to the immuno-stimulatory characteristic of the phytochemicals they contain. Researchers have shown that flavonoids may have an influence on the immune system (Aherne *et al*., 2017). The aqueous and

ethanol extracts of all the plants were found to contain the same general compounds, including saponins, alkaloids, tannins, phenols, and tannins. It was revealed that steroids, terpenoids, and anthraquinones were notably lacking in both extracts. Thus, the presence of alkaloids may explain the extract's ability to reduce parasitaemia (Idowu *et al*., 2018).

4.2.3 Acute toxicity

Assessment of toxicity is crucial in the pharmaceutical and food businesses because it reveals the potential physiological and pathological outcomes of dosing. Mammalian anatomy and physiology could be compared to the toxic effects seen in animal models. In this research, plant extracts were given an acute toxicity test (LD50), and the results indicated that even at the maximum dose (5000 mg/kg body weight), the extracts were not deadly. Acute toxicity testing results showed no observable morphological, behavioural, or major body weight changes during the first 24 hours. This study's findings indicate that the extract's fatal dosage is more than or equal to 5000 mg/kg. However, Ogwal-Okeng *et al*. (2018) found that African walnut (*Tetracarpidium conophorum*) root-bark extracted with methanol is fatal at dosages exceeding 2,000mg/kg body weight, therefore these findings contradict that claim. Differences in the plant parts utilized may account for the discrepancies in these results. In their research, Ogwal-Okeng *et al*. (2018) relied on root-bark, whereas seed fruit was analyzed in this study. As a result of greater bioaccumulation of toxicants from the soil, plant roots are often more toxic than other sections of the plant. When the plant is cultivated in polluted soil, such that which contains oil or heavy metals, the toxicity increases dramatically. Ofori-Attah *et al.* (2012) evaluation of the acute toxicity of lemon grass (*Cymbopogon citratus*) aqueous leaf extract using doses of 1250, 2500, and 5000 mg/kg in 12 mice reached similar conclusions. However, the current research

demonstrated that aqueous and ethanolic extracts of *Vernonia amygdalina, Tetracarpidium conophorum, Garcinia kola,* and *Cymbopogon citratus* were all quite safe to use.

4.2.4 Body weight of *Plasmodium berghei*-infected mice

Plasmodium berghei infection led to a substantial weight loss in untreated mice compared to infected mice treated with 100, 200 or 400 mg/kg of plant extracts or a placebo (5 mg/kg) (Table 4.4). Possible causes include an increase in metabolic rate and feed conversion efficiency, decreased appetite, and a subsequent reduction in food intake. Malaria infection has been linked to lower body weight and slower development, according to research published by Sowunmi *et al*. (2017). Hypoglycemia caused by malaria infection has been proposed as a cause of the reduced body weight seen in mice by Basir *et al*. (2012). It was found that the average body weight of mice in all treatment groups rose both before and after infection. It's possible that the plant extracts plus the placebo caused a rise in hunger. Because the mice were able to eat as much as they wanted while still maintaining a high level of feed conversion efficiency, this is consistent with the findings of Okunade *et al*. (2014), who found that plant extracts provide a decent supply of protein and energy to animals. All of these support the claim made by Craig and David, (2019) that nutrients in the food are necessary for tissue synthesis and serve as reserves of energy for the body to use in its metabolic activities.

4.2.5 Packed cell volume (PCV) of *Plasmodium berghei*-infected mice

The malaria parasite affects the erythrocytic phase, causing a decrease in red blood cells (RBCs) by haemolysis and, ultimately, a decrease in PCV. Packed cell volume values dropped across the board in infected and uninfected mice, which may have been caused by anaemia brought on by the pleocytolytic activity of *Plasmodium* on both infected and

uninfected red cells (Naria and Xiuli, 2012). This is consistent with the findings of White, (2018), who found that malaria infection leads to bone marrow dyserythropoiesis and the lysis of both infected and uninfected erythrocytes. The PCV value of the positive control was considerably ($p>0.05$) greater than that of the animals given either the water or alcohol extracts or the untreated controls (Table 4.7). The combination of extracts administered to the animals resulted in statistically significant increases that were comparable to the positive control group. High PCV levels were found at the higher dosages of the extracts that resulted in the highest percentage of clearance. This demonstrates how improved malaria suppression may lead to improved PCV in treated animals.

4.2.6 Suppression of parasitaemia in *Plasmodium berghei*-infected Mice

Due to the parasite's great sensitivity to conventional malaria treatments like chloroquine, artesunate, etc., *Plasmodium berghei* has been employed in predicting treatment results of any probable anti-malaria therapy. Commonly used for anti-malaria screening, the curative test is a standardised test that calculates the proportion of parasitaemia that has been inhibited (Momoh *et al*., 2014). This research found that *Vernonia amygdalina* ethanolic extracts were the most effective against *P. berghei*, however the aqueous and ethanolic extracts of *Tetracarpidium conophorum*, *Garcinia kola*, and *Cymbopogon citratus* also showed modest activity (Table 4.10). There was a dose-dependency in the therapeutic effects of the various extracts. The gold standard treatment (arthemether + lumafantrine) was the most effective in terms of curing the disease. However, the value was comparable to that seen with a 100 mg/kg/day dosage of bitter leaf (*Vernonia amygdalina*) extract. The lowest dosage of the ethanolic extract (100 mg/kg/bw) showed the greatest therapeutic efficacy of the plant extracts. In addition to the potential for toxicity at the highest dosages,

saturation of the plasma protein binding sites may also explain the extracts' pharmacokinetics. Previous research into the phytochemistry of the plant carried out by Alara *et al.* (2017) suggests that this may be the case, providing a potential explanation for the extracts' action. The technical explanation for the dose-dependent inactivity is that it is due to insolubility. This went against the results found by Onwusonye and Uwakwe, (2019), who found that the root bark of plant extracts had the greatest curative effect at a lower dose. Parasite densities were significantly lower in the treated groups compared to the untreated groups. Consistent with the findings of Matsuoka *et al*. (2020), the current study confirmed that treatment with a conventional anti-malarial medicine reduced parasitaemia in *P. berghei*-infected mice to an undetectable level. *Vernonia amygdalina* contains phytochemicals, which might explain why both aqueous and ethanolic leaf extracts of the plant have antimalarial action. Secondary metabolites were comparable across aqueous and ethanolic extracts, with the exception of more abundant saponins in the latter. In comparison to the placebo, the proportion of parasitaemia was considerably lower in the groups that were given escalating dosages of the combined extract, which included the positive control medication arthemether+lumafantrine combination. Although there was no significant difference in parasite clearance activity between the combined extracts at the administered doses, there was a clear advantage in selecting the combined extracts of bitter leaf and lemon grass, which showed a percentage of suppression (72%) comparable to that of the positive control group (100%).

4.2.7 Mean survival time of *Plasmodium berghei*-infected mice

The median survival rate (MSR) is a measure of how long individuals live after receiving a certain therapy for an illness. Animals infected with *P. berghei* and given either the

conventional medication or aqueous or ethanolic extracts of *Vernonia amygdalina*, *Tetracarpidium conophorum*, *Garcinia kola*, or *Cymbopogon citratus* exhibited MST that was significantly greater than infected and untreated mice (Table 4.12). This shows that the conventional medicine, as well as the aqueous and ethanolic extracts of the plant samples, were successful in reducing the parasitemia in the infected mice. Chloroquine had the greatest MST, followed by *A. boonei* stem extract, which was consistent with the results of a research comparing the genotoxicity and anti-plasmodial activity of stem and leaf extracts of *A. boonei* in mice infected with malaria conducted by Babamale *et al*. (2017).

4.2.8 Haematological indices

Indicators of treatment effectiveness against plasmodial infection often include haematological indices such as platelet count, total white blood cell count, red blood cell count, packed cell volume (PCV), and haemoglobin (Hb) level. Consistent with earlier research carried out by Eledo and Izah, (2018) and Kotepui *et al*. (2019) in both humans and rodents, there was a substantial drop in the whole haematological status (PCV value, Hb concentration, RBC and WBC counts) of the negative control group in the present investigation (Table 4.13). Malaria parasite invasion of erythrocytes, mechanical destruction of parasitized red cells, and use of Hb as nutrition source during erythrocytic stage of parasite life cycle may all contribute to a decrease in RBC count, PCV and Hb concentration of the group of mice treated with 400 mg/kgbw of *Vernonia amygdalina* and *Garcinia kola* extracts. Malaria parasites in the host may have caused these haematological abnormalities because they cause vascular adhesion by releasing adhesion molecules on the surface of parasitized erythrocytes. It also causes parasitized and non-parasitized

erythrocytes to adhere, which increases RBCs being destroyed during splenic clearance (White, 2018).

According to Muriithi *et al*. (2019), plant extracts possess erythropoietin promoting activity and phytochemicals that slow down the natural process of oxidative breakdown of erythrocytes because of the observed increase in RBCs and its indices (Hb, PCV, MCV, MCH and MCHC and PLT) in the experimental mice treated with 100 mg/kgbw and 200 mg/kgbw of *Vernonia amygdalina*, 100 mg/kgbw and 200 mg/kgbw of *Cymbopogon citratus* and all the treatment doses of *Tetracarpidium conophorum* compared with the negative control group (infected and not treated). Mice with malaria had their haematological indices improve after receiving the extracts, demonstrating that the extracts counteracted the disease's effects. This ameliorating action raises the possibility that one or more of the extract's phytoconstituents are haematopoietic. This may also imply that the motivation to maintain haematological equilibrium in the host was enhanced after the malaria parasite was eliminated. All of these findings corroborate those of Olafadehan *et al*. (2020), who found that when compared to a positive control group, animals fed bitter leaf (*Vernonia amygdalina*) extracts had significantly higher levels of key haematological parameters such as red blood cell count, haemoglobin, platelet count, monocyte count, and lymphocyte count. As shown by White's comparable results, it's likely that anaemia is to blame for the negative group's mice having lower RBC, PCV, MCH, MCV, and MCHC levels.

Malaria infection leads to haemolysis of infected and uninfected erythrocytes and bone marrow dyserythropoiesis, making it a leading cause of anaemia. This is consistent with the findings of Kotepui *et al*. (2015), who found that haemoglobin levels were dramatically

lowered in cases with high parasitemia. Balogun *et al*. (2019) provide a plausible explanation for this phenomenon, suggesting that haemoglobin degradation occurs while the malaria parasite multiplies inside the erythrocytes of infected hosts.

Mice in the negative control group showed a reduction in mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin concentration (RBC), which is similar with the findings of Asangha *et al.* (2017). In order to combat the malaria parasite, it is reasonable to anticipate an increase in white blood cell (WBC) counts in both the extract-treated and the conventional drug-treated groups. African walnut (*Tetracarpidium conophorum*) methanolic extracts may have immune-boosting characteristics, as shown by an increase in white blood cells and differential leukocytes counts in the test animal (Muriithi *et al*., 2019). Also, Muriithi *et al*. (2019) note that extract may have stimulatory effects on platelet production, leading to a significant increase in platelet probably by enhancing thrombopoietin's secretion, which is consistent with the increased platelet counts for extract-treated groups compared with normal control. An increase in neutrophils in the absence of malaria suggests that these cells get activated in response to the stress or excitement brought on by the presence of malaria parasites, and are thus able to eliminate them by phagocytosis. This is consistent with the finding of Aitken *et al*. (2018) that neutrophils are involved in the initiation and control of the immune response. The low eosinophil counts across all groups in this research may be explained by the fact that eosinophils do not play a substantial role in *P. berghei* infection, but they may provide some protection against malaria by inducing the parasite's death. The immune-stimulating and enhancing properties of bitter leaf (*Vernonia amygdalina*) extract may explain why monocyte counts were higher in the extract-treated groups. The presence of alkaloids in

bitter leaf (*Vernonia amygdalina*) suggests that it may be used as analgesics, anti-malaria, and stimulants, as suggested by Amaya and Stephen, (2018). Monocytes regulate parasite burden and aids in host defence, according to Amaya and Stephen, (2018).

4.2.9 Histopathology of experimental animals

Since the liver and kidney are key sites of metabolic activity, the influence of the extracts on their histoarchitecture was also detected in mice treated with the extracts. Histological analyses of infected mice demonstrated that the hepatocytes were restored from congestion caused by brown pigmentation (haemozoin pigment), demonstrating the usefulness of these plant extracts in conventional therapy of malaria. Liver histological analysis demonstrates that bitter leaf (*Vernonia amygdalina*) and African walnut (*Tetracarpidium conophorum*) aqueous leaf extracts considerably mitigated malaria-related liver damage. To reduce the severity of liver damage in mice with malaria, these plant extracts were given. In the photomicrographs of the livers of the treated groups given plant extracts, no endemic inflammation of the hepatocytes was seen.

Whitten *et al*. (2017) revealed that severe cases of malaria in the untreated infected group who were given just distilled water showed alterations in the liver. It was discovered that Kupffer cells were engulfing cellular debris and malaria pigments (haemozoin) in the sinusoids. Olayode *et al*. (2015) verified the presence of heavy infiltrates of inflammatory cells and found minor vascular congestion in the hepatic portal vein. Parasite-laden Kupffer cells and haemozoin were found in modest numbers in the sinusiods of plant extract-treated infected mice, and there was also some minor inflammation in the portal zone. There was no clogging of the portal vein in the liver.

Consistent with the findings of Anyaechie, (2019), who showed that the active ingredient Irodin A isolated from bitter leaf leaves is toxic to causative strains of malaria, the number of inflammatory cellular infiltrates in liver sections of infected rats treated with extract of bitter leaf (*Vernonia amygdalina*) decreased, indicating that the infection was resolving. Infected mice given an extract of bitter kola (*Garcinia kola*) had significant congestion in the portal vein and different liver areas were filled with haemozoin, and their sinusoids contained parasite-laden Kupffer cells and cellular debris. Mild sinusoidal Kupffer cell activation and the presence of haemozoin pigments were seen in the liver sections of infected individuals treated with aqueous leaf extract of walnut (*Tetracarpidium conophorum*). Few cellular infiltrates were seen, suggesting that the plasmodial infection was clearing up. No obvious venous congestion in the liver was seen in the portal vein.

Liver slices from the lemon grass (*Cymbopogon citratus*) treatment group exhibited decreased inflammatory cellular infiltrates, minor congestion of the sinusoids with Kupffer cells, and the presence of haemozoin pigment in a few spots. Not a single instance of portal vein enlargement was identified. A decrease in liver damage associated with malaria infection was seen in the groups provided with the extracts of *Vernonia amygdalina*, *Tetracarpidium conophorum*, *Garcinia kola* and *Cymbopogon citratus*, supporting the findings of Saganuwan *et al*. (2014). The kidneys of all groups showed normal glomeruli and interstitium but enlarged renal tubules and cortices after receiving the plant extract; kidney architecture was intact, and no signs of acute or chronic damage were seen. Dilated tubules and larger glomeruli were seen in kidney tissue samples taken after administration of plant extracts (Adedapo *et al*., 2019). Hepatocytes were found to be abnormally big after administration of plant extracts, and the liver's architecture was unclear, unlike in healthy

livers, where the hepatocytes are organized in a plate around the central vein. However, previous research using mice has shown that *M. oleifera* leaf aqueous extract causes hepatocyte necrosis and portal tract inflammation (Olafadehan *et al*., 2020).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Alkaloids, phenolic compounds, saponins, tannins, flavonoids and terpenoids were found in both the aqueous and ethanolic extracts of *Vernonia amygdalina*, *Tetracarpidium conophorum*, *Garcinia kola*, and *Cymbopogon citratus*. The highest concentration of phytoconstituent was found in *Vernonia amygdalina* extracts.

Both aqueous and ethanolic plant extracts showed antimalarial promise by decreasing parasite numbers in infected mice. The lowest dosage of bitter leaf ethanolic extract (100 mg/kg/bw) showed the greatest curative efficacy of the plant extracts.

The combined plant extracts have more antiplasmodial activity than the individual plant extracts. Although there was no significant difference in parasite clearance activity between the combined extracts at the administered doses, there was a clear advantage in selecting the combined extracts of bitter leaf and lemon grass, which showed a percentage parasite suppression (72%) comparable to that of the positive control group (100%).

The lethal dosage of the plant extracts is more than 5000mg/kgbw, which is consistent with standards set by the Organization for Economic Cooperation and Development.

There was a notable improvement in haematological indices of the experimental mice. As the number of red blood cells (RBCs) and their indicators (Hb, PCV, MCV, MCH, MCHC, and PLT) were found to rise in the test animals, it became clear that the plant extracts included erythropoietin-promoting activity and phytochemicals that inhibited the body's own oxidative destruction of RBCs. The improved haematological indices in the mice also

demonstrated that the extracts alleviated the haematological status changes brought on by malaria parasites.

Histological analysis of infected mice demonstrated that the hepatocytes were restored from congestion caused by brown pigmentation (haemozoin pigment), demonstrating the usefulness of these plant extracts in conventional therapy of malaria. The glomerulus of all the experimental animals is normal, but the renal tubule with cortex seems dilated after receiving the plant extract. The preserved architecture of the tubules and interstitium which showed no signs of acute or chronic damage, indicates a kidney that has not been harmed.

The results of this research provide credence to the traditional usage of *Vernonia amygdalina*, *Tetracarpidium conophorum*, *Garcinia kola* and *Cymbopogon citratus* for the prevention and treatment of malaria. The combined extracts of *Vernonia amygdalina* and *Cymbopogon citratus* had the highest antiplasmodial activity among the screened plants thus showing the synergistic effects of combined active plant parts. Therefore, *Vernonia amygdalina* can be used singly or in combination with *Cymbopogon citratus* or *Tetracarpidium conophorum* to treat malaria.

5.2 Recommendations

1. The active components may help pave the way for the discovery of a new class of powerful antimalarial drugs, therefore they need more testing.
2. The results of this study indicate that the plants may contain a wealth of new antimalarial drugs; hence, bioassay-guided fractionation is required to isolate the components of the extract responsible for the aforementioned biological activities.
3. The safety profile of these plant extracts has been established, other research should focus on evaluating different *Plasmodium* species.

4. Further research is necessary since the exact molecular mechanism(s) of action of the extracts and their active components are not known.

5.3 Contribution to Knowledge

The research revealed the presence of phytoconstituents (alkaloids, flavonoids, saponins and tannins) responsible for antiplasmodial activity in all the extracts and also established the non-toxic nature of these extracts. The PCV and body weight of infected mice increased from 36.3±0.02% to 41.7±0.02% and 27.55±0.01g to 31.02±0.02g respectively when the combined extracts of *Vernonia amygdalina* and *Cymbopogon citratus* was administered.

The administration of 100 mg/kg and 200 mg/kg ethanolic extract of *V. amygdalina* resulted in 67% and 65% parasite suppression respectively and the combined aqueous extracts of *V. amygdalina* with *C. citratus* and *T. conophorum* gave the parasite suppression of 72% and 71% respectively. Hematological studies indicated improved blood parameters as there was a noticeable increase in RBCs and its indices (Hb, PCV, MCV, MCH and MCHC and PLT) in the experimental mice treated with 100 mg/kgbw and 200 mg/kgbw of *Vernonia amygdalina*, 100 mg/kgbw and 200 mg/kgbw of *Cymbopogon citratus* and all the treatment doses of *Tetracarpidium conophorum*. A decrease in RBCs, PCV and Hb concentration was also noticed in the group of mice treated with 400 mg/kgbw of *Vernonia amygdalina* and *Garcinia kola* extracts. Histological studies revealed hepatoprotective and anti-inflammatory effects of the extracts. The liver histological analysis demonstrated that *Vernonia amygdalina* and *Tetracarpidium conophorum* aqueous leaf extracts considerably mitigated malaria-related liver damage.

This study thus revealed that *V. amygdalina* when administered singly and also when combined with *C. citratus* and *T. conophorum* has high antiplasmodial activity. These findings open new avenues for potential antimalarial drug development and highlight the holistic therapeutic potential of these plant extracts beyond their antiplasmodial activities.

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