# EFFECTS OF CO-ADMINISTRATION OF PROMETHAZINE AND ARTEMETHER-LUMEFANTRINE IN *PLASMODIUM berghei berghei* INFECTED MICE

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**ZARIA, NIGERIA**

**AUGUST, 2016**

## DECLARATION

I declare that the work in this dissertation entitled 'Effects of co-adminstration of Promethazine and Artemether-Lumenfantrine in *Plasmodium berghei berghei* infected mice' has been carried out by me in the Department of Pharmacology and Therapeutics. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Ebunoluwa Bonire ALEMIKA _____ _____

Signature Date

## CERTIFICATION

This dissertation entitled 'EFFECTS OF CO-ADMINISTRATION OF PROMETHAZINE AND ARTEMETHER-LUMENFANTRINE IN PLASMODIUM berghei berghei INFECTED MICE' by Ebunoluwa Bonire ALEMIKA meets the regulations governing the award of the degree of Master of Science in Pharmacology of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

| | | |
|---|---|---|
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## ABSTRACT

Malaria is the 2nd leading cause of death from infectious diseases in Africa, after HIV/AIDS. It is a major public health problem in Nigeria where it accounts for most cases of hospital visits and deaths than any other country in the world. This study evaluated the effects of co-administration of promethazine and Artemether-Lumenfantrine in mice infected with *Plasmodium berghei berghei.* The mice were grouped into six groups of 6 mice each. Group 2 – 6 were inoculated with *Plasmodium berghei berghei* groups. Drug administration was carried out orally and for 5 days, 72 hours after parasite inoculation and parasitaemia seen. The mice were sacrificed on the 8th day. Blood samples were collected and organs harvested for haematological and histopathological evaluations. Parameters evaluated include average parasitaemia inhibition, haematological indices, organ-body weight ratio and liver transaminases. The liver, kidney and heart were subjected to histological evaluation. The result showed that promethazine alone had no significant parasitaemia inhibition but the co-administration of promethazine (both 25 mg/kg and 50 mg/kg) and artemether-lumenfantrine gave a marginal reduction but not a significant one compared to Artemether-lumenfantrine alone. Promethazine showed a significant reduction in Haemoglobin, Pack cell volume, RBC, and increase in neutrophils compared to Artemether-Lumenfantrine alone, but no effects compared to the infected control. Its co-administration of promethazine (25 mg/kg) with Artemether-Lumenfantrine gave a marginal reduction, but not a significant difference in Hb, PCV and RBC. The 50mg/kg dose of promethazine co-administered with artemether-lumenfantrine gave lower values of PVC, Hb, RBC than the 25mg/kg promethazine dose plus artemether-lumenfantrine. The liver transaminases were significantly ($p \leq 0.05$) increased for Promethazine co-administered with artemether-lumenfantrine compared to Artemther-lumenfantrine alone, although the values of AST, ALT and ALP were still mostly within the normal ranges ( AST-20-298U/l, ALT-17-80U/l, ALP-30-110U/l). Histology sections of the liver showed slight increase in hepatic congestion and lymphocyte hyperplasia, renal sections showed no significant changes while the heart sections showed slight lymphocyte hyperplasia.

The study showed that co-administration of Promethazine and Artemether-lumenfantrine possesses no advantage over Artemether-Lumenfantrine alone and it has tendencies of toxicity with continuous use.

## ABBREVIATIONS

| | |
|---|---|
| ALT | Alaninie Tranaminase |
| AST | Aspartate Transaminase |
| ALP | Alkaline Phosphatate |
| A-L | Artemether-Lumenfantrine |
| HB | Haemoglobin |
| PCV | Pack Cell Volume |
| RBC | Red Blood Cells |
| WBC | White Blood Cells |
| PCR | Polymerase Chain Reaction |
| WHO | World Health Organisation |
| HIV | Human Immuno-Deficiency Virus |
| AIDS | Acquired Immuno-Deficiency Syndrome |
| FMOH | Federal Ministry Of Health |
| ACT | Artemisinin Combination Therapy |
| GPI | Glucose Phosphate Isomerase |
| RDT | Rapid Diagnostic Test |
| RCEAD | Rolling Circle Enhanced Activity Detector |
| ITN | Insecticide Treated Net |
| LLIN | Long Lasting Insecticide-Treated Net |
| IPT | Intermittent Preventive Therapy |
| UNICEF | United Nations Children's Emergency Fund |
| PCT | Parasite Clerance Time |
| LYM | Lymphocyte |
| NEU | Neutrophils |

| | | |
|---|---|---|
| H & E | Haemotoxylin And Eosin | |
| NADPH | Nicotinamide Adenine Di-Nucleotide Phosphate | (Reduced Form) |
| G6PD | Glucose-6-Phosphate Dehydrogenase | |
| ULN | Upper Limit Of Normal | |
| CDC | Centre For Disease Control | |

# CHAPTER ONE

## 1.1INTRODUCTION

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected mosquitoes. It is a disease caused by a protozoan parasite of the genus *Plasmodium* transmitted to humans from infected female *Anopheles* mosquitoes (Weekley and Scoot-smith, 2013).

Malaria is transmitted exclusively through the bites of *Anopheles* mosquitoes. The intensity of transmission depends on factors related to the parasite, the vector, the human host, and the environment. Malaria is an acute febrile illness, in which symptoms appear seven days or more (usually 10–15 days) after the infective mosquito bite in a non immune individual. The first symptoms – fever, headache, chills and vomiting – may be mild and difficult to recognize in malaria. If not treated within 24 hours, *P. falciparum* malaria can progress to severe illness often leading to death. Children with severe malaria frequently develop one or more of the following symptoms: severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria. In adults, multi-organ involvement is also frequent. In malaria endemic areas, persons may develop partial immunity, allowing asymptomatic infections to occur. Malaria detection currently depends largely on microscopy and rapid diagnostic test (Peipei *et al*., 2014) but it suffers poor sensitivity in scenarios of low level parasitemia of below 10 parasites/µL (Weiss,1995). However, polymerase chain reaction (PCR) based assays have been developed for the detection and identification of malaria parasites which have proven to be more specific and sensitive than conventional microscopy, it has been able to detect as few as one parasite/µL of blood(Singh *et al*., 1999).

## 1.2Statement of Research

In 2013, malaria caused an estimated 584, 000 deaths (with an uncertainty range of 367, 000 to 755,000), mostly among African children. According to the estimates, released in December 2014, there were about 198 million cases of malaria in 2013 (with an uncertainty range of 124 million to 283 million) and an estimated 584 000 deaths (with an uncertainty range of 367, 000 to 755, 000). Malaria mortality rates have fallen by 47% globally since 2000 and by 54% in the WHO African Region (WHO, 2014).WHO estimates that in 2015, malaria caused 214 million clinical episodes and 438,000 deaths. Over the years, different categories of plasmodia specie (*P. falciparum, p.ovale, p. vivax,* and *p. malaria)* evolved with several newly observed mechanisms of resistance. (Ogbona and Uneke, 2008;Simba *et al.*, 2010). Thirty countries in Sub-Saharan Africa account for 90% of global malaria deaths.Nigeria, Democratic Republic of Congo (DRC), Ethiopia, and Uganda account for nearly 50% of the global malaria deaths.Malaria is the 2$^{nd}$ leading cause of death from infectious diseases in Africa, after HIV/AIDS (WHO, 2013). Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world (FMOH, 2012). Malaria is a risk for 97% of Nigeria's population. The remaining 3% of the population live in the malaria free highlands.There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria. This can be compared with 215,000 deaths per year in Nigeria from HIV/AIDS (WHO, 2013).Malaria is the 3rd leading cause of death for children under five years worldwide, after pneumonia and diarrheal (WHO, 2013). It accounts for about 60% of all out patient attendance and 30% of all hospital admissions (FMOH, 2008). Resistance to antimalarial medicines is fast becoming a great problem. Resistance of *P. falciparum* to previous generations of medicines, such as chloroquine

and sulfadoxine-pyrimethamine (SP), became widespread in the 1970s and 1980s, undermining malaria control efforts and reversing gains in child survival.

Antimalarial drug resistance – the ability of the malaria parasite to survive drugs – first became a global problem in the 1960s when the parasite developed resistance to chloroquine, the then widely-used antimalarial. Resistance first emerged in the Greater Mekong sub region and later spread to Africa, triggering a dramatic increase in malaria-related illness and death – particularly among children. Malaria is the most important parasitic infection in people and has become a priority for the international health community as it is also the focus of several new initiatives. One of such new initiatives includes the development of artemisinin combination therapies (ACTs) proven to be the most effective first-line strategy for the treatment of uncomplicated *P. falciparum* malaria, presently eliciting a high degree of resistance to conventional antimalarial drugs (Wongrischanalai *et al*., 2002; Breman *et al.,* 2004).

Today the treatment of choice is artemisinin-based combination therapies (ACTs),artemether-lumefantrine is an effective ACT known popularly under the brand names COARTEM® or RIAMET® and is used majorly in Africa, due to its high efficacy against the parasite. The standard recommended dosage regimen for the drug is a fixed 6-dose regimen (24tablets; 480mg artemether and 2880 mg lumefantrine, given over

3 days) as the oral first line treatment with the substance to be administered at 0, 8, 24, 36, 48 and 60 h of the treatment course (Van-Vugt,*et al.,* 1999).

Resistance to artemisinins, the core component of the combination artemeter-lumenfantrine, has now been identified in Cambodia, Myanmar, Thailand, and Viet Nam (WHO, 2014). National efforts to contain resistance have had some impact, but urgent action is needed to fully eliminate resistant strains of the parasite and to ensure

that ACTs remain effective. An estimated mortality rate of 0.7 – 2.7 million is caused by malaria with more than 300million cases of malaria illness occurring each year and more than 90 percent of these deaths are in Africa (David and Peter, 2004). This has resulted to a serious substantial economic and social hardship.

Promethazine is an antihistamine that acts by competing with histamine for H-1 receptor sites on effector cells. This H-1 antagonist is also used as adjunct therapy in the treatment of malaria in english-speaking West African countries. The drug is given as an antiemetic with chloroquine to prevent or alleviate chloroquine-associated pruritus, (Sowunmi *et al.*, 1998). It is also given with arthemeter- lumenfantrine commonly, at a dose of 5.0–10 mg is given simultaneously, or just prior to administration of chloroquine in children with falciparum malaria in Nigeria. In adults, a daily dose of 25 mg is well tolerated.

## 1.3 Justification

According to WHO, Malaria is the single most important cause of ill health, death and economic loss in Sub-Saharan Africa). The management of malaria is complicated because the parasites that cause malaria are resistant to most of the safest and cheapest first line treatments developed so far (WHO, 2013).

There is an urgentneed for discovery of new antimalarial combinations. Malaria vaccines such as FMPI/ASO2 that targets falciparum malaria protein1 (FMP1) has shown no promising effect in the prevention of malaria (Ogutu *et al.,* 2009) and RTS,S/ASO2A a pre-erythrocytic stage malaria vaccine only provides partial protection against the infection (Alonso *et al*., 2006), yet attempts are ongoing to develop a perfect vaccine for prevention of malaria. Thus, chemoprophylaxis and chemotherapy remains the primary methods in prevention and treatment of acute

attacks (Sanon *et al*., 2013). The rising levels of resistance to antimalarial drugs which may occur due to inappropriate administration (Gilbert, 2009) and the control of malaria is becoming nearly impossible with recent detection of artemisinin resistance on Cambodia (WHO, 2012). High levels of resistance have being seen in some antimalarial drugs such as chloroquine and also with insecticides, even with the resistance, there are few available and affordable alternatives (Harriet *et al*., 2013). It is therefore necessary to find effective and affordable alternative treatment in which the parasite will not be resistant.

Nausea and vomiting is one of the major symptoms of malaria and in other for it to be well managed, it is a common practice clinically to prescribe promethazine for nausea and vomiting alonside artemether-lumenfantrine an antimalaria, this is very common particularly in children. Also with the developing trend of resistance to artemeter-lumenfantrine, it is of importance to know what added advantage promethazine has on artemeter-lumenfatrine aside its antiemetic properties. The potentiating effect of promethazine on amodiaquine has been established in a study(Olalubi*et al.*,2011).

This study aims at determining the effects of promethazine, an antihistamine co-administered with artemeter-lumefantrine, an artemisinin based combination antimalarial therapyin*P. berghei* infected albino mice.

## 1.4 Aim and Objectives

### 1.4.1 Aim

The aim of this research is toevaluate the effects of promethazine on the antimalarial activity of artemeter-lumenfantrine in *P. berghei* infected albino mice.

### 1.4.2 Specific Objectives:

The specific objectives of this study are:

1. To determine the effects of promethazine on parasitaeamia level in *P. berghei* infected mice.
2. To determine the effects of co-administration of promethazine and artemether-lumenfantrine on haematological parameters in *P. berghei*infectedalbino mice
3. To evaluate the histopathological effects and other changes of co-administration of promethazine and artemether-lumenfantrine on *P. berghei* infected albino mice.

## 1.5 Research Hypothesis

1. Promethazine will increase the antimalarial activity of artemether-lumenfantrine when co-administered in *P. berghei* infected albino mice

or

2. Promethazine will not increase the antimalarial activity of artemether-lumenfantrine when co-administered in *P. berghei* infected albino mice

# CHAPTER TWO

## *2.0 LITERATURE REVIEW*

### *2.1 Malaria*

Malaria is an infectious disease that is wide spread in tropical and sub-tropical regions of the world. As of 2015, there were more than 200 million malaria cases and more than 400,000 malaria deaths worldwide. More than 3 billion people are still at risk of infection in 97 countries and territories. The disease remains heavily concentrated in low-income countries of sub-Saharan Africa (WHO, 2015). According to World Health Organization, 80% – 90% of these occur in Africa alone with children and pregnant women being the most susceptible. Malaria kills about 584,000 people each year and a child dies of malaria in every 30 seconds in sub Saharan Africa(WHO, 2013)

Malaria is a disease of high mortality and morbidity. It is an infection with an enormous public health problem. The causative organism belongs to the genius plasmodium species which are:

*P. falciparum, P. vivax, P. malariae, P. ovule,*and*P.berghei.*

The *plasmodium malariae* causes "quartian malaria" form which the fever occurs every 72 hours (every 4 days). P *vivax*causes tertian malaria where the fever occurs every 48 hours. P *falciparum* causes sub-tertian malaria where the fever peaks in less than 48 hours but because it invades the central nervous system, it causes malignant tertian malaria (cerebral malaria). P berghei infects rodents in the same way it affects man.

Malaria is derived from the Italian word "mal-aria" or BAD air suggesting the exhalation of marshland ALUBISM. The French term MAL derived from Latin

meaning MARSH. Malaria then is marshland fever related to the proliferation of its vector in those swampy regions (WHO, 2016).

Thirty countries in Sub-Saharan Africa account for 90% of global malaria deaths. Nigeria, Democratic Republic of Congo (DRC), Ethiopia, and Uganda account for nearly 50% of the global malaria deaths (WHO, 2016).

In Nigeria, malaria accounts for more cases and deaths than any other country in the world. Malaria is a risk for 97% of Nigeria's population (WHO,2016), the remaining 3% of the population live in the malaria free highlands. There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria, it contributes to an estimated 11% of maternal mortality (WHO, 2016).

### 2.1.1 The Life Cycle of Plasmodium Parasite

The life cycles of all the four (4) plasmodium species are similar. Malaria infection is transmitted by a mosquito which has part of its life cycle within the vector (mosquito) while the host (man) houses the second part of the life cycle (Baer *et al.,* 2007). The phase which occur in the mosquito is the sexual phase (exogenous phase or sporogony) while the phase that occur in man is the asexual phase (endogenous or schizogony) (*Hume et al., 2003).* Infection is indicated by the bite of infected female anopheles mosquito which injects saliva containing sporozoites and anti-coagulant into the host. The sporozoites are motile and disperse within an hour from the circulation into the liver. In the liver they divide rapidly within the hepatic parenchyma – cell into pre-erytrozoites. One sporozoite may give rise to between 2,000 – 40,000 merozoites; depending on the plasmodium species (*Tan et al., 2008).* The development of merozoites in the liver cells is called exoerythrocytic or pre-erythrocytic phase (*Martinsen et al., 2008).* Not all plasmodium parasite exhibits exoerythrocytic cycle, the

pre-erythrocytic merozoites are liberated into the blood stream and invade red blood cells where they undergo a nonsexual new nucleic development to form multi-nucleic schizoites. Each erythrocyte containing these merozoites ruptures, releasing their loading parasites including pyrogens (*Chavatte et al., 2007).* This is the asexual phase of schizogomy cycle. Released merozoites can be destroyed by phargocyctic cells but those that escape the phargocytes will enter fresh red blood – to reinitiate an asexual cycle. The duration for this cycle varies from 48 hours in *P. falciparum* and *P. vivax* to 72 hours in *P. malarae (Martinsen et al., 2008).* This cycle continues as large numbers of merozoites are produced to induce fever and often signs and symptoms that characterize malaria. For some reason unknown some of the trophozoites failed to divide asexually, rather they differentiate into male and female genatocytes *(Hedrick et al.,* 2011). These gametocytes do not produce any chemical features of malaria but are active and responsible for the continuity and sexual phase of the parasite. They infect the mosquito feeding from malaria patients. When mosquito sucks the blood all other forms of the parasite are digested and destroyed in the mosquito stomach. The male gamate undergoes ex-flagelate (loses flagellal) liberating microgamates and fertilization occurs in the female gametes to give the zygote*(Martinsen et al., 2008).* They differentiate in the wall of the mosquito gut into sporozoites which finally accumulate in the insect salivary gland to complete the sexual cycle of the development. All the typical clinical symptoms and severe disease pathology associated with malaria are caused by the asexual erythrocytic or blood stage parasites. When the parasite develops in the erythrocyte numerous known and unknown waste substances such as haemozoin pigment and other toxic factors accumulate in the infected red blood cell (*Hedrick et al.,* 2011). These are dumped into the blood stream when the infected cells lyse and release invasive merozoites. The haemozoin and other toxic factors such as glucose phosphate

isomerase (GPI) stimulate macrophages and other cells to produce cytokins and other soluble factors which act to produce fever, rigors and probably influence other severe pathophysiology associated with malaria (CDC, 2004). *P. falciparum* infected erythrocytes, particularly those with mature trophozoites, adhere to the vascular endothelium of venular blood vessels walls and do not freely circulate in the blood. Where thin sequestration of infected erythrocytes occurs in the vessels of the brain and is believed to be a factor in causing the severe disease syndrome known as cerebral malaria, which is associated with high mortality.Anaemia is also associated with malaria infection and is frequently severe in children and pregnant women infected with *P. falciparum*. Severe anaemia can also be associated with *P. Vivax* infections. Macrophages not only clear infected erythrocytes but also phagocytize and destroy uninfected red blood cells during malaria infections. Active malaria infections also through unknown mechanism induce bone marrow dyscrasias and suppress normal development (CDC, 2004). Intravascular haemolysis does not appear to be a major contributor to malarial anaemia except in the pathological state known as black water fever (CDC, 2004).

### 2.1.2 *Plasmodium berghei*

*Plasmodium berghei* is a unicellular parasite (protozoan) that infects mammals other than humans. *P. berghei* is one of the four *Plasmodium* species that have been described in African murinerodents, the others being:

- *Plasmodium chabaudi*
- *Plasmodium vinckei*
- *Plasmodium yoelii*

These are not of direct practical concern to man or his domestic animals. The interest of these parasites is that they are practical model organisms in the laboratory for the experimental study of human malaria. This species was first described by Vincke and Lips in 1948 in the Belgian Congo (Otto *et al.,* 2014).

Studies have shown that *P. berghei* can produce a severe degree of infection presented by the high degree of parasitaemia followed by death 6-7 days' post infectionand antimalarial drugs like chloroquine and pyrimethamine are effective in the treatment or prevention of malaria in *P. berghei infected* laboratory mice (Protus *et al*., 2014).

### 2.1.3 Incidence and Prevalence

Malaria transmission occurs in five WHO regions (Africa,South east Asia, Western pacific region, eastern Meditarrien region and parts of America). Globally, an estimated 3.2 billion people in 95 countries and territories are at risk of being infected with malaria and developing the disease and 1.2 billion are at high risk (> 1 in 1,000 chance of getting malaria in a year). According to the world malaria report 2015, there were 214 million cases of malaria globally in 2015 (uncertainty range 147-303 million) and 438, 000 malaria deaths, (range 236, 000-635, 000) representing a decrease in malaria cases and deaths of 37% and 60% since 2000 respectively (WHO, 2016)

Malaria is a major public problem in the world particularly sub-saharan Africa (WHO, 2014). The burden was heaviest in the WHO African region having 88% of the global malaria cases and 90% of the malaria deaths globally of which children under 5 years accounted for more than two- thirds of all the deaths.

World Health Organization report that the factors responsible for the incidence and prevalence of malaria in Africa includes failure in health system, drug resistance,

population mobility climate changes, war and in some cases unplanned developmental activities (WHO, 2010).

In Nigeria, malaria is constantly the most common cause of attendance at health care facilities and ranges among the top three causes of deaths (FMOH, 2012). The actual incidence of mortality rate of malaria is unknown due to incomplete and irregular reporting. However, other areas of the world have not been spared of the increasing ravage of malaria (WHO, 2014). At community level the magnitude and severity of malaria varies with the intensity of transmission and level of endemicity in the area (FMOH, 2012).

The wide spread of malaria in Nigeria still remains a major contributory factor and leading cause of infants and child deaths (WHO, 2015). Malaria adversely affects the psychological well-being of individuals and families and is a major cause of socio-economic deprivation (WHO, 2010). It is the most common cause of absenteeism in school and work places in Nigeria (FMOH, 2012). The situation of malaria in Nigeria is sustained by the presence of very efficient vectors of malaria as well as favourable factors such as temperature ($20^0$C – $30^0$C) rainfall (mean monthly rainfall of greater than 10$cm^3$ relative humidity (rated higher than 60%) and topography calibration of less than 2000cm above sea level (FMOH, 2012).

Malaria remains a major public health and development challenge in Nigeria. It is estimated that in Nigeria children under the age of five years have 2 to 4 attacks of malaria every year and appropriately 50% of the adult population experience at least one episode of malaria per year (FMOH, 2012). Malaria currently accounts for nearly 110 million clinically diagnosed cases per year. 60% of these are out-patients visits and 30% hospitalization; also an estimated 300,000 children die of malaria each year with

up to 11% of maternal mortality. In addition to the direct health impact of malaria, there is also sex, social and economic effects on our communities and country as a whole. About 132 billion naira is lost to malaria annually for treatment cost, prevention and loss of man power (FMOH, 2012).

According to CDC (2004), factors that determine the occurrence of malaria are those that influence the three components of malaria life cycle:

- Anopheles mosquitoes must be present.
- Humans must be present.
- Malaria parasites must also be present.

Climate is the key determinant in the geographical distribution and seasonality of malaria as it influences the three components of the life cycle. Rainfall creates the breeding sites where *anopheles'* eggs are deposited, and lava and pupae develop into adulthood, a process that takes approximately 9-12 days in tropical areas. Once adult mosquitoes have emerged, the ambient temperature, humidity and rains will determine their chances of survival. Female *anopheles'*mosquitoes must survive long enough after being infected to successfully transmit the malaria parasites theyharbour to complete their growth cycle (extrinsic cycle), which takes 9-12 days at $25^0$C. Warmer ambient temperatures shorten the duration of the extrinsic cycle, thus increasing the chances of transmission. On the other hand, below a minimum ambient temperature ($15^o$C for *P.vivax*, $20^o$C for *P.falciparum*), the extrinsic cycle cannot be completed and malaria cannot be transmitted. This explains in part why malaria transmission is greater in warmer areas of the globe (tropical and semitropical areas and lower attitudes), particularly *P. falciparum*. It has been speculated that current trends of global warming may increase the geographic range of malaria and may be responsible for malaria epidemics (CDC 2004).

Climate also determines human behaviours that may increase contact with *anopheles'*mosquitoes between dust and dawn, also the period when the*anopheles* mosquitoes are most active. People tend to sleep outdoors in hot weathers and are discouraged to use bed nets. During harvest seasons, agricultural workers might sleep in the fields without protection against mosquito bites.

Biological characteristics (in born and acquired) and behavioural traits can influence an individual's malaria risk and, on a larger scale, the intensity of transmission in a population.

### 2.1.4 Signs and Symptoms of Malaria

The symptoms of malaria generally begin 7 – 15 days after a bite from an infected female anopheles' mosquito. It can also be contacted by blood transfusion from an infected individual.

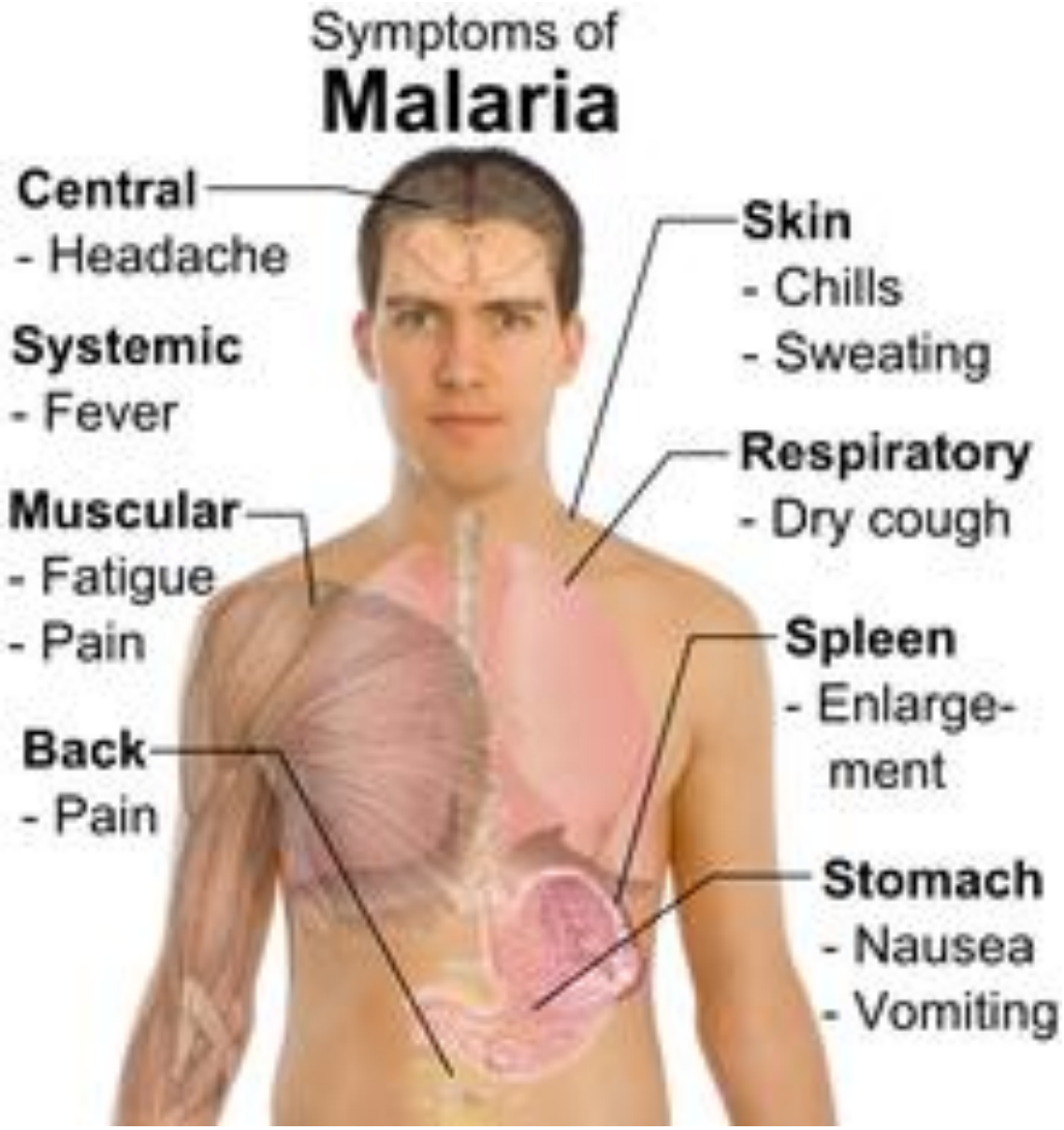



**Fig 2.1: Symptoms of Malaria**

Adapted from www.whoreport2014.com/malaria/signsandsymptoms

The parasite first attacks the red blood cell which subsequently burst and releases waste and toxins along with merozoites. Fever develops as the immune system responds to the toxin in the blood. An attack begins with chills and shivering, soon followed by a high fever, these attacks usually leave individual exhausted. Headache, nausea and vomiting and aches may also accompany the fever and chills.

### 2.1.5 Complications of malaria

Malaria can cause a number of life threatening complications, the following may occur:

- Swelling of the blood vessels of the brain or cerebral malaria.
- An accumulation of fluid in the lungs that causes breathing problems, or pulmonary oedema.
- Organ failure of the kidneys, liver, or spleen.
- Anaemia due to the destruction of red blood cells.
- Low blood sugar.

### 2.1.6 Diagnosis of Malaria

Prompt and accurate diagnosis of malaria is part of effective disease management and if implemented effectively, it helps to reduce unnecessary use of anti-malarial drugs. High sensitivity of malaria diagnosis is important in all setting especially for the most vulnerable groups such as young children, and pregnant women in which the disease can be rapidly fatal.

The diagnosis of malaria is based on clinical criteria supplemented by the detection of parasite in the blood parasitological or confirmatory diagnosis. Since Charles Laveran first visualised the malaria parasite in blood in 1880, the mainstay in diagnosis has been microscopic examination of blood (WHO, 2010).

Rapid diagnostic test (RDT) using immune-chromatographic tests (dipsticks, cassettes format) is a very fast method that takes only 15-20 minutes, it can be done inthe field, it requires only a drop of blood, and can detect 2 types of antigens.

A new test makes it possible to diagnose malaria using a single drop of blood or saliva. The test was developed by researchers at Aarhus University in Denmark and can detect

very low levels of an enzyme produced by the plasmodium parasite. This new method uses a technology called Rolling circle Enhanced Enzyme Activity Detection (REEAD). This detection technique is much more time and cost effective (Abdullahi, 2014)

Clinical diagnosis alone has very low specificity and in many areas, parasitological diagnosis is not currently available. The decision to provide anti-malarial treatment in these settings must be based on the prior probability of the illness being malaria. One needs to weigh the risk of withholding anti-malarial treatment from a patient with malaria against the risk associated with anti-malarial when given to a patient who does not have malaria.

### 2.1.7 Prevention and Control of Malaria

Methods used to prevent the spread of disease or to protect individual in area where malaria is endemic, includes prophylactic drugs, mosquito eradication and the prevention of mosquito bite.

The objective of malaria control programmes ranges from reducing the disease burden and maintaining it at a reasonably low level, to eliminating it from a defined geographical area and ultimately to eradicating it globally (WHO, 2015).

After repeated infections, people who live in regions where, malaria is prevalent developed limited immunity to the disease. This partial protection does not prevent them against the most serious effect of the infection. They generally developed mild form of the disease that does not last long and is unlikely to be fatal.

Malaria prevention and control attempts include:

- Vector control programme

Malaria was once common in United States and Southern Europe but vector control programs in conjunction with monitoring and treatment of infected humans eliminated it from those regions. It also includes better sanitation, use of pesticides and draining of wet land breeding grounds.

- Indoor residual spraying (IRS).

  Indoor residual spraying is also effective in endemic areas. Indoor residual spraying is the practice of spraying insecticides on the walls of homes in malaria affected areas. After feeding, many mosquito species rest on a nearby surface while digesting the blood meal. So if the walls of dwellings have been coated with insecticides, the resting mosquitoes will be killed before they can bite another victim, to transfer the malaria parasites

- Insecticide-treated mosquito nets (ITN) and long lasting insecticide- treated net (LLITN).

  Mosquito nets and bed clothes have greatly reduced the infection and transmission of malaria

- Sterile insect technique involving transgenic/ genetically modified mosquitoes that is malaria resistant. A newer strategy by researchers in Italy in 2002, involves the development of genetically modified non-biting mosquitoes
- Laser to kill flying insects
- Education of the populace on better sanitation, use of ITN, use of insect repellents, life-cycle of the malaria parasite, use of antimalarial drugs to prevent transmission (IPT) in pregnant women, infants and school children and the correct use of drug combination therapy
- Vaccination

### 2.1.8 Vaccine Research

Vaccines for malaria are under development without an effective one yet available. RTS,S/AS01 is the most advanced vaccine candidate against the most deadly form of human malaria, *P. falciparum*. A Phase III trial began in May 2009 and has completed enrolment in 2011 with 15, 460 children in the following seven countries in sub-Saharan Africa: Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, and the United Republic of Tanzania (WHO, 2015).

There are two age groups in the trial:

1) Children aged 5-17 months at first dose receiving only the RTS,S/AS01 vaccine; and

2) Children aged 6-12 weeks at first dose who receive the same malaria vaccine co-administered with pentavalent vaccines in the routine immunization schedule. Both groups receive 3 doses of RTS,S/AS01 vaccine at 1 month intervals (WHO, 2015). The final Phase III results were published in April 2015. The vaccine will be evaluated as an addition to, not a replacement for, existing preventive, diagnostic and treatment measures (Greenwood, 2015). The need for long-lasting insecticidal nets, rapid diagnostic tests and artemisinin-based combination therapies will continue if RTS,S/AS01 becomes available and is use (WHO, 2015). Immunity or more accurately tolerance does occur naturally, but only in response to repeated infection with multiple strains of malaria.

## 2.2 Classification of Antimalarial Drugs

Antimalarial drugs are classified into two (2):

1. Therapeutic classification
2. Chemical classification

### 1.Therapeutic Classification

A. Causal prophylaxis: (Primary tissue schizonticides)

- Destroy parasite in liver cells and prevent invasion of erythrocytes
- Primaquine, proguanil

B. Suppressives Prophylaxis:

- Suppress the erythrocytic phase so that clinical malaria does not appear and thus attack of malarial fever can be used as prophylactics
- Chloroquine, Proguanil, Mefloquine, Doxycycline

C. Clinical cure: erythrocytic schizonticides

- used to terminate an episode of malarial fever

I, Fast acting high efficacy, can be used alone

- Chloroquine, quinine, mefloquine, atovaquone, **artemisinin**

II, Slow acting low efficacy drugs, these are used in combination

- Proguanil, pyrimethamine, sulfonamides, tetracyclines

D. Radical curatives:

- Eradicate all forms of P.vivax & P.ovale from the body, That is they attack the exo-erythrocytic stage of the parasite in the liver, the hypnozoites.
- Supressive drugs + hypnozoitocidal drugs
- For vivax: primaquine 15 mg daily for 14 days

E. Gametocidal:

- Destroy gametocytes and prevent transmission
- Primaquine, **artemisinin** – against all plasmodia
- Chloroquine, quinine

**2.Chemical Classification**

A. 4 aminoquinolines:

- Chloroquine, Hydroxychloroquine, Amodiaquine, Pyronaridine

B. 8 aminoquinolines:

- Primaquine, Tafenoquine, Bulaquine

C. Cinchona alkaloids:

- Quinine, Quinidine

D. Quinoline methanol:

- Mefloquine

E. Biguanides - Proguanil, Chlorproguanil

F. Amino Alcohol- Lumenfantrine, Halofantrine

G. Mannich base- Pyronaridine

H. Sesquiterpenes lactones e.g Artemether, Artesunate, Arteether

I. Napthoquinone e.g Atovaquone

J. Di-amino-pyrimidine e.g Pyrimethamine

K. Tetracyclines e.g Tetracycline, Doxycycline

L. Sulphonamides and Sulphone e.g Sulphadoxine, Dapsone, Sulfamethopyrazine

## 2.3 Guidelines for the Treatment of Malaria

Malaria is a serious and potentially fatal disease, particularly in the case of falciparum parasites and especially in non-immune individuals. It is such a problem in many parts of the world that a global partnership; Roll Back Malaria, WHO, United nation Development Programme, UNICEF and the World Bank projected to significantly reducing the World's malaria burden by 2015 but these was not achieved. For effective malaria treatment, prompt diagnosis is crucial. Treatment is with blood schizonticides, selected with due regards to the prevalence of specific patterns of drugs resistance in the area of infection (WHO, 2015). In the case of*P. vivax,P.ovale* and *P. malariae*, subsequent treatment should involve the use of amodiaquine, due to cross resistance between chloroquine and amodiaquine. If either of these options is or becomes

unsatisfactory then mefloquine or quinine may be used. Quinine is usually given with tetracycline or doxycycline to ensure a high cure rate (WHO, 2014).

Increasingly however, *P. falciparum* is developing resistant to these conventional therapies, hence WHO recommended the use of combination therapy which consists of artemisinin derivation (artemisinin – based combination therapies also known as ACTs). The following combination therapies are recommended:

- Artemether – lumenfantrine
- Artesunate – plus amodiaquine
- Artesunate plus pyrimethamine – sulfadoxine (in area where susceptibility to pyrimethamine – sulfadoxine remains high).
- Artesunate plus mefloquine (normally reserved for areas of low transmission).

Additional ACTs recommended by WHO for accelerated development are piperazine – dihydroartemisinin, chlorproguanil-dapsone-artesunate (Dacart) and pyrimidine-artesunate. In severe or complicated falciparium malaria including cerebral malaria, parenteral treatment is required to produce adequate blood concentration as quickly as possible. The importance of achieving this tissue schizoiticide (e.g. artemether-lumafantrine) is needed where it is considered appropriate to prevent relapse.

Anti-malarials are generally given orally. Although in order to obtain a rapid response in patients with severe to complicated falciparum malaria, it may be necessary to give parenteral therapy initially, the patient switched to oral therapy as soon as it is feasible.

### 2.3.1 Treatment of *Falciparum* Malaria

In most parts of the world P. *falciparum* is now resistant to chloroquine. Therefore, apart from the rare circumstances of exposure in one of the few remaining areas of

chloroquine sensitivity, it is not suitable for treatment (WHO, 2013). In U.K uncomplicated malaria falciparum is treated with one of the following quinine and (if quinine resistance is known or suspected) followed by either pyrimethamine – sulfadoxine or (if sulphadoxine – pyrimethamineresistant) doxycycline (or clindamycin in children).

Mefloquine; atovaquone – proguainil, and Artemether – lumefantrine are often used.

For malaria endemic areas (like Nigeria) where there is chloroquine resistance, WHO recommended ACT as the treatment of choice in uncomplicated malaria (WHO, 2015).

Therapeutic concentrations of anti-malaria drugs as early as possible have been emphasized at the site of action in order to achieve quick therapeutic effect (WHO, 2013). Chloroquine should be given if the infection is known to be sensitive to it.Chloroquine is still the first line treatment for *P.vivax* and *P. ovale*, while primaquine can be used to treat liver stage parasites of *P.vivax*, in areas of low transmission if adherence is guaranteed.

In choloroquine resistant malaria or where sensitivity to chloroquine is not known, quinine is usually given intravenously starting with a loading dose. Intravenous artesunate or intramuscular artemether may used if parenteral quinine or artemisinin derivatives are not available. Patients of all ages need to be closely monitored while undergoing parenteral therapy and treatment switched to orally administered anti-malaria as soon as the patients conditions permits. When there are only minimal health care facilities or parenteral therapy is not possible, artemisinin or artesunate suppositories may be given (Lengeler *et al.*, 2010). The nasogastric route may also be used. Supportive therapy in patients with severe or complicated malaria needs to be

directed at reducing hyper-pyrexia, controlling convulsion, maintaining fluid balance and correcting hypoglycaenia.

### 2.3.2 Treatment of Benign Malaria

Benign malaria which are usually caused by *P. vivax* or less commonly by *P. ovale* or *P. malariae*, can be debilitating but are usually less severe than falciparum malaria (Robert *et al.*,2009). Chloroquine is still the drug of choice but chloroquine alone is adequate for *P. malariae* infections, the cases of malaria caused by *P. vivax or P. ovale* or when a radical cure with a tissue schizoiticides usually primaquine is used to avoid the risk of relapse (caused by the presence of later hypnozoides), months or years after the primary infection (WHO, 2014). Radical cure is also appropriate for patient living in an endemic area since re-infection is likely and therefore WHO recommends that it should be limited to patients residents in areas where transmission is very low or absent and to those treated during an epidemic (WHO, 2014). Other patients should simply be treated with a further course of chloroquine in the event of relapse or re-infection. *P.vivax* cases that fail to respond to chloroquine may be treated with quinine or mefloquine.

### 2.3.3 Treatment of Malaria During Pregnancy

Malaria is specifically dangerous during pregnancy and the seriousness of the disease usually outweighs any potential risk from treatment (WHO, 2013). Although fetal abnormalities have been associated with the use of high doses of chloroquine, its extensive clinical exposure suggests it is safe, its use may be limited, however, due to

resistance, quinine may be used for chloroquine resistance malaria but care should be taken so that the patients do not become hypoglycaemic.

Artemisinin combination therapies are the drugs of choice for the treatment of uncomplicated malaria in the second and third trimester; data regarding their use in the first trimester is at present limited (WHO, 2015). Intermittent treatment with pyrimethamine – sulphadoxine as single dose in the second and third trimester may reduce the risk of severe anaemia in late pregnancy; in malaria endemic areas, although there are some concerns over emerging resistance (Barnes *et al.,* 2008).

### 2.4 Artemisinin-based Combination Therapy (ACTs)

Successful malaria control depends greatly on treatment with efficacious anti-malarial drugs. Countries have a National Malaria Treatment Policy,that specifies drugs for treatment of both uncomplicated and severe malaria, malaria in pregnancy and what to do if first line treatment fails.

As drug resistance develops to existing drugs, new ones need to be introduced.

For *P. falciparum,*the use of two or more drugs with different modes of action in combination is now recommended to provide adequate cure rate and delay development of resistance.

Currently, artemisinin-based combination therapy (ACT) is recommended for the treatment of *P. falciparum* malaria. Fast acting artemisinin-based compounds are combined with a drug from a different class, companion drugs include lumefantrine, mefloquine, amodiaquine, sulfadoxine/pyrimethamine, piperaquine and chlorproguanil/dapsone. Artemisinin derivatives include dihydroartemisinin, artesunate

and artemether. Implementation of the recommendation to use ACTs is limited by the small number of available and affordable co-formulated anti-malarial drugs, but most countries are now starting to implement this regimen. A co-formulated drug is one, in which two different drugs are combined in one tablet; this is important to ensure both drugs are used.

The benefits of ACTs are their high efficacy, fast action and the reduced likelihood of resistance developing. In order to make best use of them, it is critical to address issues of delivery, access and cost.

### 2.4.1 Artemether-lumefantrine

Artemether-lumefantrine is an ACT prequalified by the WHO for efficacy, safety and quality, approved by Swissmedic in December 2008 (WHO, 2009).
Artemether/lumefantrine was the first fixed-dose artemisinin-based combination therapy recommended and pre-qualified by WHO for the treatment of uncomplicated malaria caused by *P. falciparum*. It has been shown to be effective both in sub-Saharan Africa and in areas with multi-drug resistant *P. falciparum* in Southeast Asia. It is currently recommended as first-line treatment for uncomplicated malaria in several countries. However, its complex treatment regimen of two doses daily for three days could affect adherence.
Both artemether and lumefantrine are blood schizonticides with complementary pharmacokinetics and dissimilar modes of action, and hence provide synergistic anti-malarial activity. (Novartis, 2014). Lumefantrine is a highly effective, long acting, oral erythrocytic schizonticide related to mefloquine.

Highly lipophilic onset delayed, peak at 6 hours, it is available as fixed dose combination, 80 mg artemether with 480 mg lumefantrine twice daily for 3 days.Artemether is rapidly eliminated from plasma with a half-life of two to three hours, whereas lumefantrine is eliminated more slowly with a half-life of three to six days

The combination provides a high long-term cure rate after a short treatment course (Norvatis, 2014) and thus provides rapid clearance of parasitemia and most malaria-related symptoms, coupled with prevention of recrudescence.

Adverse effects: In adults, the most frequently reported adverse reactions of the combination were headache, anorexia, dizziness, and asthenia. In children, the adverse reactions were pyrexia, cough, vomiting, anorexia, and headache. Most adverse reactions are mild and do not lead to discontinuation of medication.

## 2.5The Challenge of Drug Resistance

The development of resistance to each anti-infective therapy is inevitable — antimalarial therapy is no exception. The frequency of occurrence of a resistant *P. falciparum* parasite in an infected patient is estimated to be 1 in $10^{10}$. In a severe malaria patient, the parasite burden can rise to $10^{12}$-$10^{13}$, which means that there could be up to 1,000 resistant parasites in one patient alone. This is a fact that will need to be considered in the treatment of malaria until the parasite can be eradicated. As mentioned, the malaria eradication campaign of 1955 failed partly due to drug resistance, primarily to chloroquine (CQ) and later to the replacement drug sulfadoxine-pyrimethamine (Wongsrichanalai *et al.*, 2002).

Today the treatment of choice in the majority of malaria cases is ACT. The key to artemisinin's activity is believed to lie within the presence of its peroxide bridge

(Wongsrichanalai*et al.,* 2002). Artemisinin derivatives, such as artesunate, artemether, and dihydroartemisinin, are short-acting antimalarial agents that kill parasites more rapidly than previous generation antimalarials. They are able to achieve 10,000-fold reductions in parasite burden per asexual cycle and as a result fever clearance time is reduced to 32 hours compared to days. While highly effective, the concern is that if used alone, parasites resistant to artemisinin will develop and spread, making the disease potentially untreatable. Therefore, in 2006 the WHO produced new treatment guidelines stipulating that the treatment of choice for uncomplicated *P. falciparum* malaria should be fixed-dose ACT, where artemisinin is protected against the emergence of resistance by the presence of a second medicine (WHO, 2006).

Over the last 5 years more than 300 million patients have been treated with ACTs, which is a tremendous achievement. However, with such large treatment numbers and the fact that 28 countries still allow the use of artemisinin monotherapy (against the advice of the WHO), resistance is almost bound to occur. Resistance to the partner drug has always been a hazard, but is one that is manageable now that we have a range of ACTs — either launched or submitted to stringent regulatory authorities. Artemisinin resistance, however, is a much bigger issue, since there are fewer alternatives. The first signs of emerging resistance have been reported recently in Southeast Asia (Dondorp *et al.,* 2009). An almost two-fold increase in parasite clearance time (PCT) to artesunate has been reported in Pailin, western Cambodia. For the moment, ACTs are still effective. But the recent reports of delayed PCT are an early warning that treatment failure could be just a few years down the line. The key questions we have at this stage are, how long before resistance to artemisinin spreads and which other antimalarials might also fall prey to the same strain of resistance?

Unfortunately, whether artemisinin resistance will spread in the future is not a question of if, but when. It is difficult to put a timeframe on this; chloroquine resistance took over 10 years to dominate Africa (Wongsrichanalai *et al.,* 2002), yet still remains an effective medicine in some parts of the world. Within the next 10 years, however, it is likely that a few things will change. We have received the first sign - increased PCT. The second will be the occurrence of parasites that don't respond to artemisinin derivatives at all. The third sign will be that 28-day response to therapy is reduced. WHO defines a successful drug as one that maintains 95% cure rate at 28 days. From the point that this occurs, current estimates suggest that it might be over at least a decade before the artemisinins lose their efficacy globally.

The second question, of which other antimalarials could possibly fall prey to the same strain of resistance, is less straightforward to answer than the first. Once the chemical structure of artemisinin was known, many groups worked hard to make synthetic artemisinins — free from the vagaries of agriculture. Rather than making the whole molecule, most groups concentrated on synthesizing the chemically reactive group — the endoperoxide. Two of these molecules are now in clinical development — Arterolane [Ranbaxy/Medicines for Malaria Venture (MMV)] in Phase III, and OZ439 (MMV) in Phase II, (although Arterolane is presently in use). One key experiment is to test these medicines in patients from areas where the potential emergence of artemisinin resistance has been reported. These studies are currently being designed. Fortunately for now, such patients are rare, but this makes the clinical studies very difficult and slow to carry out. Further investigations are also underway to determine the mode of action of resistance to the artemisinins, which will also help shed light on the question posed. Again, it is interesting that no one has yet been able to produce a stable parasite line which is resistant. This suggests that multiple mutations are needed to produce

artemisinin resistance in the parasite, and that these have a relatively high cost to the parasite. However, the focus for a new medicine has to be one which is active against all the potential drug-resistant strains, and with a completely new mechanism of action. Such molecules are now being discovered by MMV and our partners, but even with a successful clinical program, these remain 7 or more years away from being launched into the community. In recent years, parasite resistance to artemisinins has been detected in 5 countries of the Greater Mekong subregion: Cambodia, Lao People's Democratic Republic, Myanmar, Thailand and Viet Nam. Studies have confirmed that artemisinin resistance has emerged independently in many areas of this subregion. Most patients are cured when treated with an ACT if there is no resistance to the partner drug.

However, in parts of Cambodia and Thailand, *P. falciparum* resistance to both artemisinin and partner drugs (multi-drug resistance) has developed.

There are concerns that *P. falciparum* malaria in Cambodia and Thailand is becoming increasingly difficult to treat, and that multi-drug resistance could spread to other regions with dire public health consequences. Consequently, WHO's Malaria Policy Advisory Committee in September 2014 recommended adopting the goal of eliminating *P. falciparum* malaria in this subregion by 2030. WHO launched the Strategy for Malaria Elimination in the Greater Mekong Subregion (2015–2030) at the World Health Assembly in May 2015, which was endorsed by all the countries in the subregion.

## 2.6Malaria Elimination

Malaria elimination is defined as interrupting local mosquito-borne malaria transmission in a defined geographical area, typically countries; i.e. zero incidence of

locally contracted cases. Malaria eradication is defined as the permanent reduction to zero of the worldwide incidence of malaria infection caused by a specific agent; i.e. applies to a particular malaria parasite species.

On the basis of reported cases for 2013, 55 countries are on track to reduce their malaria case incidence rates by 75%, in line with World Health Assembly targets for 2015(WHO 2014). Large-scale use of WHO-recommended strategies, currently available tools, strong national commitments, and coordinated efforts with partners, will enable more countries – particularly those where malaria transmission is low and unstable – to reduce their disease burden and progress towards elimination.

In recent years, 4 countries have been certified by the WHO Director-General as having eliminated malaria: United Arab Emirates (2007), Morocco (2010), Turkmenistan (2010), and Armenia (2011). In 2014, 13 countries reported 0 cases of malaria within their own borders. Another 6 countries reported fewer than 10 cases of malaria.

## 2.7Promethazine

Promethazine is a neuroleptic medication and first generation antihistamine of the phenothiazine family.

Mechanism of action: It is an antihistamine that acts by competing with histamine for $H_1$ receptor sites on effector cells.

Pharmacokinetics: Promethazine is well absorbed from the gastrointestinal tract. Clinical effects are apparent within 20 minutes after oral administration and generally last four to six hours, although they may persist as long as 12 hours. Promethazine is metabolized by the liver to a variety of compounds; the sulfoxides of promethazine and

N-demethylpromethazine are the predominant metabolites appearing in the urine (Rxlist, 2015).

Uses: It is a potent histaminic $H_1$ antagonist with additional anti-emetic and sedative/calming properties. This $H_1$ antagonist is also used as adjunct therapy in the treatment of malaria in English-speaking West African countries. It is also used in the symptomatic treatment of allergic conditions of the upper respiratory tract and skin; sensitisation reactions to medicinal agents or foreign proteins; anaphylactic reactions and as a hypnotic/sedative agent. For pre-medication, it is used for its sedative/calming effect, anti-emetic action and anti-secretory effect. (it provides clinically useful sedative and antiemetic effects)

Adverse effects: drowsiness, dry mouth, fatigue, constipation, seizures, chest discomfort, euphoria.

# CHAPTER THREE

## 3.0 Materials and Methods

### 3.1Materials

The following are the materials used in this study:

#### *3.1.1 Animals*

The animals used in this study were male and female albino mice (6 – 8 weeks old) weighing 18 – 22 grams. The animals were obtained from the animal house of the Department of Pharmacology and Therapeutics,Ahmadu Bello University, Zaria. The mice were kept in cages floored with wood shavings and placed on standard rodent feed with free access to tap water. All animals were distributed into groups (1-6) and each group was made up of 6 animals. The mice were used in accordance with the National Institute of Health (NIH) Guide for the care and use of laboratory animals(NIH,1996).

#### 3.1.2 *Plasmodiumberghei*

The mice infected with *Plasmodium berghei* were obtained from the Department of Microbiology, National Medical Research Institute Yaba, Lagos.Fresh mice were inoculated every 2 weeks to preserve the plasmodium parasite. The re-infected mice were kept in the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

#### 3.1.3 Drugs and Chemicals

- Promethazine, (Avomine$^{(R)}$) manufactured by Sanofi Aventis Pharmaceuticals.
- Artemether-lumenfantrine (manufactured byNorvatis Pharma AG, Basel, Switzerland, was obtained from Norvatis Pharma NigeriaFormalin (2.5%) was manufactured by BDH

Pooled, U.K and was obtained from the Department of Pharmacology and Therapeutics, ABU Zaria.

- Normal saline solution (0.9%), manufactured by Dana Pharmaceuticals Ltd, Nigeria was obtained from ABUTH Shika.

All drugs were administered orally.

### 3.1.5 Equipments and Glasswares

- Avery balance (W and T, Avery ltd; Birmingham England
- Top loading balance (P 162 Gallenkamp)
- Microscope (Olympus)
- Vernier caliper (Triple brand, model number 04818048.)
- Mortar and pestle(England)
- Disposable hypodermic insulin syringe and needle (1ml)
- Plastic cages, animal water bottles and wood shavings
- Dropping pipette (Pyrex, England)
- Measuring cylinder(Pyrex, England)
- Microscopic glass slides with cover slips (Pyrex, England)

## 3.2 Methodology

### 3.2.1Inoculation of Malaria Parasite

Blood from a donor mouse infected with *Plasmodium berghei* ANKA strain with rising parasitaemia (about 20-30% infected erythrocyte) was used for this study.Fresh mice were inoculated every 2 weeks to preserve the plasmodium parasite.Blood was collectedusing a needle and syringe from the saphenous vein and transferred into a sterile bottle and mixed with normal saline in such a way that 0.2ml of blood contained approximately $1x10^7$ infected red blood cells(Phillipson, 1991).

### 3.2.2 Anti-malarial test *in vivo*

Thirty-six (36)male and female albino mice, weighing 18 – 22 g were randomly divided into six groups of six mice each. Group 1 mice, served as the negative control (and was given 0.2 mls of normal saline daily throughout the treatment days, 5 days).Groups 2-6 were inoculated intraperitoneally with $1 \times 10^7$ red blood cells (0.2ml) infected with the chloroquine resistant *P. berghei* ANKA strain on day 0 (Phillipson, 1991).

Three days (72hours) after all the mice in groups 2-6 were infected and parasitaemia observed, promethazine and artemether-lumenfantrine were administered to each of the remaining 5 groups. Drug treatment for each group is described below:

Group I: Received 0.2mls of normal saline and served as the negative control

Group II: Received0.2mls of normal saline and served as the infected control

Group III: Received10/60 mg/kg of artemether-lumenfantrine for five days

Group IV: Received 25 mg/kg of promethazine alone for five days

Group V: Received10/60 mg/kg of artemether-lumenfantrine 10/60mg/kg and 25 mg/kg of promethazine for five days

Group VI: Received10/60 mg/kg of artemether-lumenfantrine and 50mg/kg of promethazine for five days

The stock solutions of the drug samples were diluted to the desired final concentration with distilled water so that each animal received 0.2mls at the time of administration of each drug. Parasiticidal activity was assessed on days 3, 5, 7 and 8 post infection.

Blood smears were prepared to determine the parasitaemia level. About three drops of blood were collected from the tail of each mouse and smeared on a microscope slide to make a thin film.The smears were fixed in absolute ethanol, stained with 10% Geimsa stain and examined microscopically. The parasitaemia was determined by counting infected erythrocytes in hundred fields, it was then divided by the total erythrocytes in the hundred fields and multiplied by one hundred. Mortality was monitored daily, until the mice were sacrificed on days 8

Inhibition of parasite growth in drug-treated group was calculated in relation to parasite growth in the infected (untreated) control group using the formular below:Average % inhibition of parasitemia =

$$\frac{\text{average \% parasitemia in control} - \text{average \% parasitemia in test}}{\text{average \% parasitemia in control}} \times 100$$

### 3.3Necropsy

All animals were sacrificed by anaesthestic chloroform, 24 hours following the last treatment on day 7 and blood was collected by cardiac puncture into heparin bottles. Plasma was separated after centrifugation at 4,500 g at room temperature for 25 minutes.

### 3.4Haematological Assay

Serum samples from both treated and control animals were analysed for Packed cell volume (PCV), Haemoglobin concentration (Hb) content, red blood cell (RBC), white blood cells (WBC), Neutrophils, Lymphocytes and Eoisinophils using automated Mindray BC-5300 Haematolog Analyzer made in China.

### 3.5Biochemical Investigations

The serum activity of Alanine aminotranferase (ALT), Aspartate aminotransaminase(AST) and Alkaline phosphatase (ALP) were determined by using an automated analyzer( Selectra Junior Spinlab 100, vital Scientific, Dieren, Netherland; spinreact, Girona, Spain) according to the manufacturers' instruction.

### 3.6Weight of the animals

The animals were weighed three (3) days after infection with *P.berghei,*on alternate days, 3,5,7 and the last day of the experiment before the animals were sacrificed. The mice were sacrificed on the fifth day of drug treatment and seven (7) days post-infection and the weights were recorded.

### 3.7Histopathological Examination

The mice were sacrificed on the 8th day post-infection,the kidneys, liver and heart were carefully excised, cleared of adhering tissues and weighed.The relative organ weight (ROW) was calculated using the formular below:

$$ROW = \frac{\text{Absolute organ weight(mg)}}{\text{Body weight of mouse on sacrifice day (mg)}}$$

The organs removed wereprocessed for embedment in paraffin wax after fixation in 10% formalin (Drury & Wallington, 1973). Sections were cut 4-5um with rotary microtone, stained and observed under photo microscope. The photomicrographs were also observed for histopathological changes.

### 3.8Statistical Analysis

All quantitative data were expressed as the mean ± standard error of mean (SEM). Statistical analysis was carried out using ANOVA and Duncan's post hoc test. Statistical difference in means between treatment and control were assessed at 95% level of significance i.e.*P*-values less than 0.05 *(*$P \leq 0.05$) were considered significant.

## CHAPTER FOUR

## 4.0 RESULTS

## 4.1 Effects of Promethazine on Antimalarial activity of Artemether-lumenfantrine on *Plasmodiumberghei berghei*Infected Mice

Analysis of the result of the treatment groups to establish significant difference in parasitaemia was conducted for each of the day and the data for post infection days 3, 5, 7 and 8 was recorded as seen in figure 4.1 below. The mean level of parasitaemia did not differ significantly on day 3 and day 5for the different combinations of the treatment administered (Fig 4.1). Significant decrease ($p<0.05$) was observed for the mean level of parasitaemia for days 7 and 8.

On days 3& 5, there was no significant decrease *(*$p<0.05$) in the mean parasitaemia level in the different groups administered with promethazine (25 mg/kg and 50 mg/kg) and artemether-lumenfantrine. However, on day 7, there was significant decrease *(*$p\leq0.05$) in parasiatemia in the artemether-lumenfantrine compared to the infected control. Promethazine at doses 25 mg/kg and 50 mg/kg co-administered with artemether-lumenfantrine produced an insignificant increase($p>0.05$) in parasitaemia,compared to artemether-lumenfantrine administered alone.

On days 7 and 8, artemether-lumenfantrine administered group produced a significant decrease ($p<0.05$) in parasitemia compared to the infected control group and the artemether-lumenfantrine plus promethazine (25mg/kg) treated group.

In general, it was observed that artemether-lumenfantrine gave a significant ($P<0.05$) average parasitaemia reduction compared to infected control group, promethazine (25 mg/kg) produced aninsignificant decrease ($p>0.05$) in average parasitaemia when compared to the infected control. However, promethazine(25mg/kg) plus artemether-lumenfantrineand the artemether-lumenfantrine plus promethazine (50 mg/kg) treated groups produced a significant decrease in parasitaemia level ($p<0.05$) compared to the infected control. There was no significant difference in average parasitaemia of the

different doses of promethazine in its combination with artemether-lumenfantrine, although the 25mg/kg dose of promethazine co-administered with artemether-lumenfantrine produced a higher average percentage inhibition than the 50mg/kg dose of promethazine co-administered with artemether-lumenfantrine (Fig 4.1).

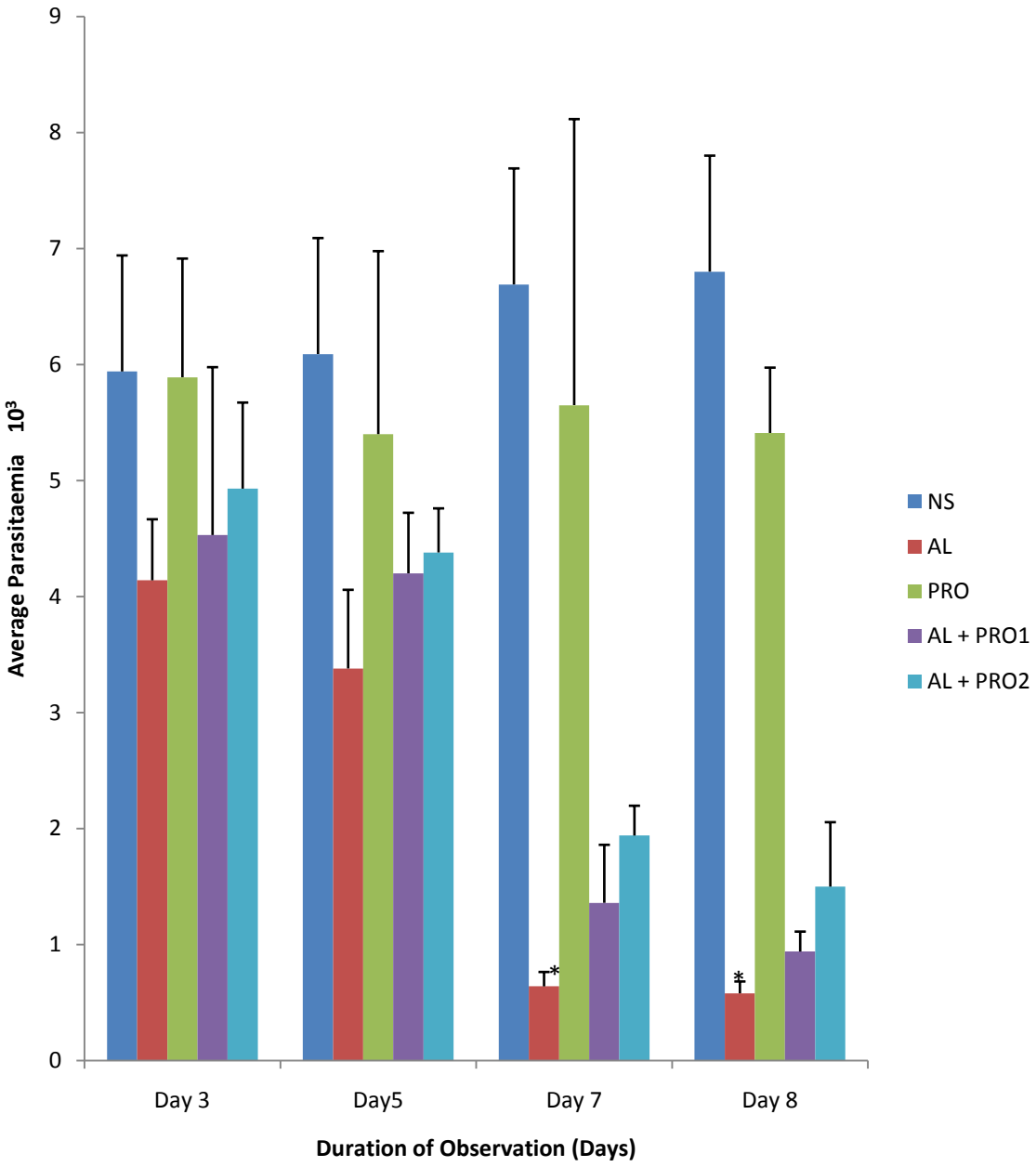



**Fig 4.1: Effects of promethazine on antimalarial activity of artemether-lumenfantrine in *P. berghei* infected mice**

* p<0.05; n=5N/S= Normal saline (0.2 ml/kg), AL=Artemether-lumenfantrine(10-60 mg/kg),

PR=Promethazine(25 mg/kg),AL+PRO1=Artemether-lumenfantrine plus Promethazine(25 mg/kg),

AL+PRO2=Artemether-lumenfantrine plus Promethaziine(50 mg/kg);

D3-=DAY 3, D5=DAY 5, D7=DAY 7, D8= DAY8

The average percentage inhibition was computed for the 8th day alongside the average parasitaemia for that day. Artemether-lumenfantrine produced a significant decrease ($p$<0.05) in average parasitaemia compared to the infected control. The A-L plus promethazine(25mg/kg) treated group and the A-L plus promethazine (50mg/kg) also produced a significant decrease ($p$<0.05) in average parasitaemia compared to the infected control group although it was not as low as A-L alone. Promethazine (25mg/kg) alone produced a significant decrease ($p$<0.05) in average percentage inhibition (Table 4.1).

**Table 4.1: The Effect of Promethazine on Antimalarial Activity of Artemether-Lumenfantrine in *Plasmodium berghei* infectedmice (eight days' post-infection)**

| Treatment | Dose(mg/kg) | Average Parasitaemia | Average percentage inhibition |
|---|---|---|---|
| **N S-A** | 0.2ml/kg | 0.00 | 0.00 |
| **N S-B** | 0.2ml/kg | 6.8±2.8 | 0.00 |
| **A-L** | 10/60 | 0.6±0.3* | 85.6±6.9 |
| **Promethazine** | 25 | 5.4±2.8 | 23.3±14.4 |
| **A-L + Pro** | 10/60 + 25 | 1.4±1.2* | 69.2±27.9 |
| **A-L + Pro** | 10/60 + 50 | 1.5±3.7* | 41.9±68.8 |

n =6, * p<0.05 ( within the group) (ANOVA, post-hoc=Duncan)

A-L = Artemether-lumenfantrine, Pro = Promethazine, NS= Normal saline

A= Normal Control (uninfected), B= Infected Control, Values presented as ±SEM

## 4.2 The Effect ofCo-administration of Artemether-lumenfantrine and Promethazine on the Haematological Parameters of *Plasmodium berghei berghei* Infected Mice

The artemether-lumenfantrine administered group produced significant ($p<0.05$) increase in haemoglobin compared to the infected control, (Table 4.2). Promethazine at a dose of 25mg/kg co-administered with artemether-lumfantrine produced a higher haemoglobin value compared to promethazine at 50mg/kg co-administered with artemether-lumenfantrine.

Promethazine (25 mg/kg) alone produced a significant ($p<0.05$) reduction in PCV values compared to the artemether-lumenfantrine treated groups.

There was no significant difference in the WBC values across the groups. This was the same for the RBC values, although promethazine treated group had the lowest value after the infected normal group. The dose of promethazine (50mg/kg) co-administered with artemether-lumenfantrine produced an insignificant decrease ($p > 0.05$)in RBC compared to the dose of promethazine at 25mg/kg co-administered with Artemether-lumenfantrine.

### 4.2: The Effect of Co-administration of Promethazine and Artemether-lumenfantrine on Haematological Parameters of *Plasmodiumberghei* Infected Mice

n=6, *p<0.05, ANOVA, Duncan post-hoc test,

| Treatment (mg/kg) | Hb(g/dl) | PCV % | WBC($l^{-1}$) | RBC ($l^{-1}$) | PTC($l^{-1}$) | N % | L % | E % |
|---|---|---|---|---|---|---|---|---|
| Unif+NS (0.2 ml/Kg) | 15.7±0.6 | 47.3±1.8 | 4.7±0.5 | 6.2±0.2 | 7.8±0.1 | 16.0±2.5 | 77.8±1.2 | 1.7±0.3 |
| Inf+NS (0.2ml/Kg) | 9.9±0.6 | 29.5±1.81 | 5.3±0.4 | 5.1±0.2 | 7.3±0.1 | 21.3±6.5 | 70.3±1.1 | 1.5±0.3 |
| Inf+AL | 14.3±0.6* | 42.5±1.8 | 5.0±0.2 | 6.1±0.2 | 7.7±0.1 | 18.5±1.3 | 79.8±1.1* | 1.7±0.3 |
| Inf+Pro 1 | 9.3±0.6 | 27.3±1.8 | 5.2±1.3 | 5.2±0.2 | 7.2±0.1 | 24.0±1.2 | 72.2±1.2 | 1.5±0.1 |
| Inf+AL+Pro 1 | 13.7±0.6* | 40.2±1.8 | 4.9±0.1 | 6.0±0.2 | 7.5±0.6 | 19.0±5.4 | 75.2±1.1* | 1.7±0.2 |
| Inf+AL+Pro 2 | 13.6±0.6* | 39.8±1.8 | 5.2±1.2 | 5.9±0.2 | 7.4±0.1 | 21.3±3.5 | 75.5±1.2* | 1.2±0.2 |

AL=Artemether-Lumenfantrine (10/60 mg/kg). Pro 1= Promethazine (25 mg/kg)

Pro 1= Promethazine (25 mg/kg) NS= Normal Saline, Hb= Haemoglobin,

PCV=Pack Cell Volume, WBC= White Blood Cells

RBC= Red Blood Cells, PTC= Platelet Cells, N=Neutrophils, L= Lymphocytes,

E= Eisinophils Inf=Infected Uninf=Uninfected

### 4.3 The Effects of Drug Co-Administration of Promethazine and Artemether-Lumenfantrine on the Body Weight of the*Plasmodium berghei berghei* Infected Mice

There was no significant difference produced in the body weight for the promethazine and artemether-lumefantrine treated groups (Table 4.3).

**Table 4.3 The Effects of Promethazine and Artemeter-lumefantrine on Body Weight of *Plasmodium berghei* Infected Mice**

| | Weight(mg) | | | |
|---|---|---|---|---|
| **Treatment** | **Day 3** | **Day 5** | **Day 7** | **Day8(last day)** |
| Unifected + NS | 19.6±2.2 | 20.7±2.5 | 21.2±2.5 | 20.5±2.3 |
| Infected + NS | 19.4±2.1 | 20.0±2.3 | 18.8±2.3 | 18.9±2.4 |
| Infected + AL | 19.0±2.0 | 20.6±2.9 | 21.1±2.3 | 21.2±2.1 |
| Infected + Pro 1 | 19.4±2.3 | 19.1±2.3 | 18.8±2.2 | 18.8±2.4 |
| Infected + AL +Pro1 | 19.6±2.1 | 20.6±2.5 | 21.2±2.1 | 21.4±2.3 |
| Infected + AL +Pro2 | 19.7±2.4 | 20.5±2.3 | 20.5±2.3 | 20.2±2.2 |

N=6, ANOVA, Duncan post-hoc test

NS=Normal Saline-0.2 ml/kg AL- Artemether-lumenfantrine, 10-60 mg/kg,

Pro1= Promethazine 25 mg/kg Pro2= Promethazine 50 mg/kg

### 4.4 The Effects of Co-administration of Promethazine and Artemether-lumenfantrine on Liver Enzymes *in P.berghe*i Infected Mice

The effect of promethazine and artemether-lumenfantrine on liver enzymes, Alanine Aminotransferase, (ALT), Aspartate Aminotransferase, (AST) and Alkaline Phophatase (ALP) in malaria infected mice was computed as presented below, (Table 4.6).There was significant difference ($p$<0.05) in ALT between the promethazine ( 25mg/kg and 50 mg/kg) and artemeter-lumefantrine treated group. The infected mice with the treatment of artemether-lumenfantrine and promethazine (50 mg/kg) had the highest ALT but were not significantly different from the infected mice with promethazine (25 mg/kg) treatment alone and those treated with artemether-lumenfantrine and promethazine (25 mg/kg).

Promethazine treated group produced a significant increase($p$<0.05) in AST compared to other treatment groups. There was an increase in the AST in the groups treated artemether-lumenfantrine and promethazine that was dose dependent in promethazine (AL + promethazine 50mg/kg was higher than AL + promethazine (25 mg/kg).

Promethazine (25 mg/kg) treated group produced a significant different increase (p<0.05) in ALP compared to the infected normal group. The promethazine (50 mg/kg) plus AL andpromethazine (25 mg/kg) plus AL treated groups produced an increase in ALP compared to the infected control group and the AL treated group but this was not statistically significant ($p$>0.05).

**Table 4.4: Effects of Co-administration of Promethazine and Artemether-lumenfantrine on Liver Enzymes in *P. berghei* Infected Mice**

| Drug treatment | ALT(units/l) | AST (units/l) | ALP (units/l) |
|---|---|---|---|
| Uninfected + NS | 40.8±1.7 | 21.5±11.5 | 66.2±6.6 |
| Infected +NS | 47.0±5.0 | 35.5±32.8 | 70.0±4.9 |
| Infected +A-L | 51.0±8.6 | 32.3±5.6 | 67.8±7.4 |
| Infected + Pro 1 | 77.8±20.8* | 55.5±32.8* | 80.0±7.6* |
| Infected + A-L+ Pro 2 | 71.5±24.2* | 45.8±13.2 | 73.2±4.5 |
| Infected + A-L+ Pro 2 | 78.0±24.9* | 47.8±11.0 | 75.1±2.5 |

N =6, SEM ± (Standard Error of Mean), *p<0.05 (within the group)

ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase,

ALP=Alkaline phosphatase

Pro1- Promethazine 25 mg/kg, A-L= Artemether-lumenfantrine 10-60 mg/kg,

NS=Normal Saline, Pro2- Promethazine 50 mg/kg

### 4.5 Effects of Co-administration of Promethazine and Artemether-lumenfantrine on Organ Weight in *Plasmodium berghei* Infected Mice

The co-administration of promethazine and Artemether-lumenfantrine produced no significant difference when compared with other treatment groups, (Table 4.3). However,there was an increase in liver and kidney weights of mice treated withpromethazine (25mg/kg) and artemether-lumenfantrine treated group and promethazine (50mg/kg) and artemether-lumenfantrine treated group (Table 4.3)

Promethazine (25mg/kg) produced an insignificant ($p \geq 0.05$) increase in thekidney weight.

**Table 4.5: The Effects of Co-Administration of Promethazine and Artemether-Lumenfantrine on Organ Weight in *P. berghei* Infected Mice**

| Treatment | Liver(mg) | Kidney(mg) | Heart(mg) |
|---|---|---|---|
| Uninf+NS | 2.0±0.1 | 0.4±0.5 | 0.2±0.1 |
| Inf+NS | 2.4±1.1 | 0.4±0.4 | 0.1±0.1 |
| Inf +A-L(10/60mg) | 2.1±0.5 | 0.4±1.1 | 0.2±0.1 |
| Inf +Pro (25mg/kg) | 2.3±1.1 | 0.5±1.1 | 0.2±0.1 |
| Inf +AL +Pro1 | 2.2±1.2 | 0.4±0.2 | 0.2±0.1 |
| Inf +AL +Pro2 | 2.2±1.0 | 0.5±1.2 | 0.2±0.1 |

n =6, ANOVA, Duncan post-hoc test

NS=Normal Saline A--L Artemether-lumenfantrinePro1=Promethazine 25mg/kg,

Pro 2= Promethazine 50mg/kg Inf=InfectedUninf=Uninfected

### 4.6 Effects of Promethazine and Artemether-lumenfantrine Co-administrationon Organ-Body Ratio in *Plasmodium berghei* Infected Mice

The organ-body weight ratio was computed using the body weight of the mice on the last day of the experiment before they were sacrificed and the organ weight of three different organs that were weighed after sacrificing the animals and carefully separating the organs, livers, heart and kidney.

Organ-body ratio of liver = weight of liver ÷ body weight of mouse

The results were computed and presented in Table 4.5.

There was no significant difference across the groups in the organ-body weight ratio

**Table 4.:6 Effects of Co-Adminstration of Promethazine and Artemether-Lumenfantrine on Organ/Body Weight Ratio in *P.berghe*i Infected Mice**

| Drug treatment(mg/kg) | Liver/body weight | Kidney/body weight | Heart/body weight |
|---|---|---|---|
| UNF +NS | 0.09±0.02 | 0.02±0.01 | 0.01±0.01 |
| Inf +NS | 0.12±0.01 | 0.02±0.01 | 0.01±0.01 |
| INF+A-L | 0.10±0.01 | 0.02±0.01 | 0.01±0.01 |
| INF+ Pro1 | 0.12±0.01 | 0.02±0.01 | 0.01±0.00 |
| INF +AL+ Pro1 | 0.12±0.01 | 0.02±0.01 | 0.01±0.01 |
| INF +AL+Pro2 | 0.12±0.02 | 0.02±0.01 | 0.01±0.00 |

n= 6, ANOVA, Duncan post-hoc test, A-L = Artemether-lumenfantrine (10-60mg/kg)

Pro1 = Promethazine 25mg/kg, Pro2= Promethazine 50mg/kg

UNF=Uninfected INF=Infected weight- mg

## 4.7 Histopathological Evaluation of the Effects Of Promethazine and Artemether-lumenfantrine on Some Selected Organs of *Plasmodium berghei* Infected Mice

Histopathological evaluation of some selected organs of mice administered promethazine and artemether-lumenfantrine showed only a few variations from the normal.

Liver sections of the normal control,(uninfected and treated with normal saline 0.2mls) showed normal histomorphology, while the infected control (treated with normal saline 0.2mls) showed necrosis (Plates I). Those treated with artemether-lumenfantrine alone (10-60 mg/kg) showed lymphocyte hyperplasia, while promethazine (25 mg/kg) treated group showed vascular congestion. The groups treated with artemether-lumenfantrine(10-60 mg/kg) and promethazine (25mg/kg) showed vaoculation and necrosis. The higher doseof promethazine (50 mg/kg) co-administrated with artemether-lumenfantrine showed more vaoculation necrosis.

Heart sections of the normal control and infected controlgroups showed normal cardiac muscles and lymphocyte respectively. The sections of the promethazine (25 mg/kg and 50 mg/kg) and artemether-lumenfantrine co-administered group showed lymphocyte hyperplasia (Plate II).

Kidney sections of the normal control showed normal glomerulus and tubules, while the infected control showed distortions of glomerulus and tubules with necrosis (Plates III).However, the sections of the mice treated with artemether-lumenfantrine and Promethazine,(25 and 50 mg/kg) showed lymphocyte hyperplasia.

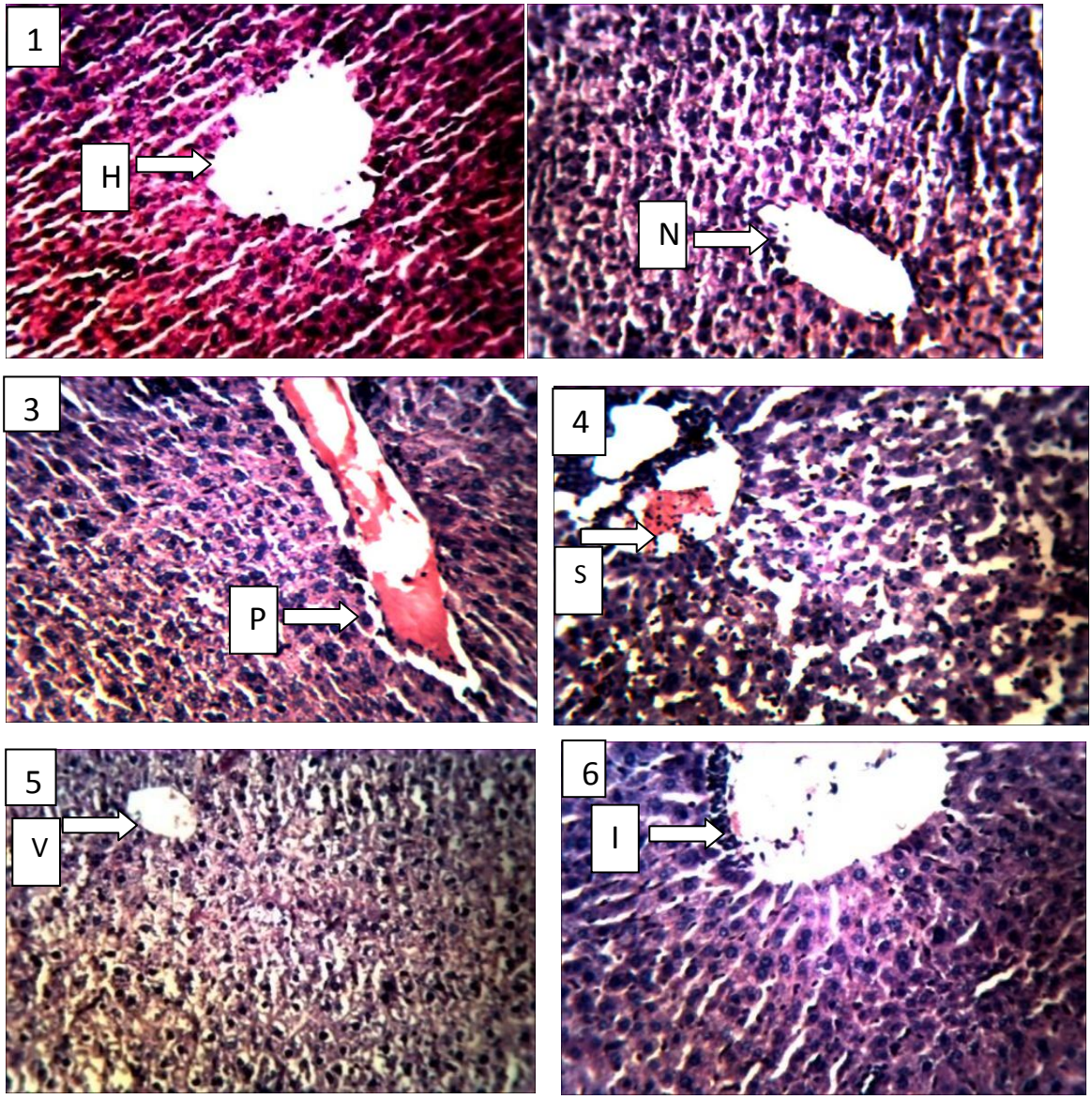



**PLATE I:** Histopathological section of liver tissue of mice treated as follows:**(**H & E stain, × 400)

**1.** Normal control mice treated with normal saline showing normal hepatic tissue, hepatocyte (**H)**,

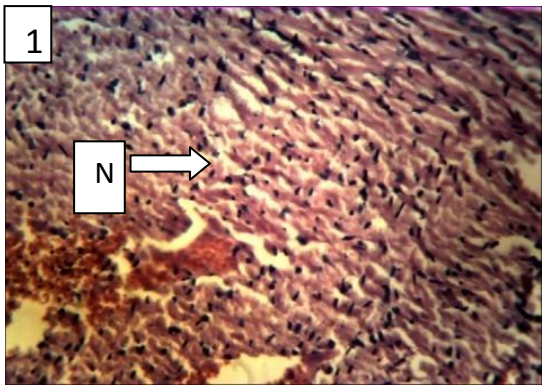
**2.** *Plasmodium berghei*infected control mice treated with normal saline showing necrosis **(N**)

**3**Artemether-lumenfantrine (70mg/kg) treated mice showing lymphocyte hyperplasia with pigmentation **(P)**

**4.** Promethazine (25mg/kg) treated group showing vascular congestion and slight pigmentation **(S)**

**5**. Mice treated with Artemether-lumenfantrine(70mg/kg) and Promethazine(25mg/kg) showing vaoculation and necrosis (**V)**

**6.**Artemether-lumenfantrine (70mg/kg) and promethazine (50mg/kg) treated group showing intense vaoculation and necrosis **(I)** × 800



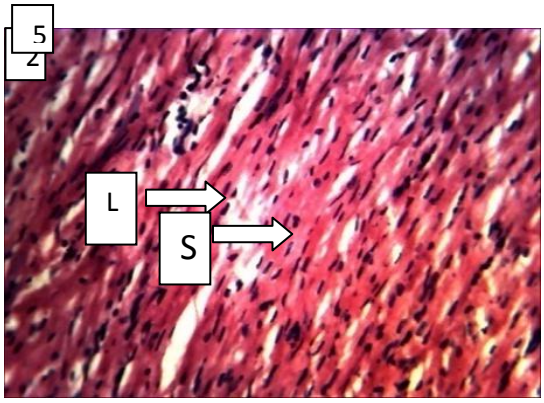




**PLATE II:** Histopathologicalsection of heart tissueof mice treated as follows: (H & E Stain. × 400

3 4

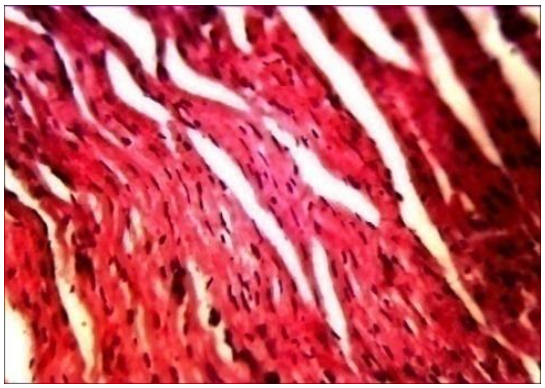
**1.** Normal control mice treated with normal saline showing normal cardiac muscle cell, **(N)**

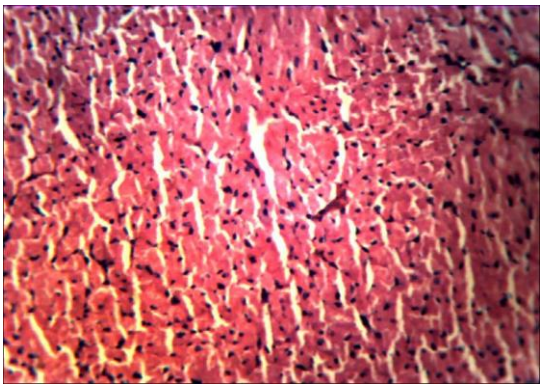
**2.** *Plasmodium berghei*infected control mice treated with normal saline showing lymphocyte**, (L)**

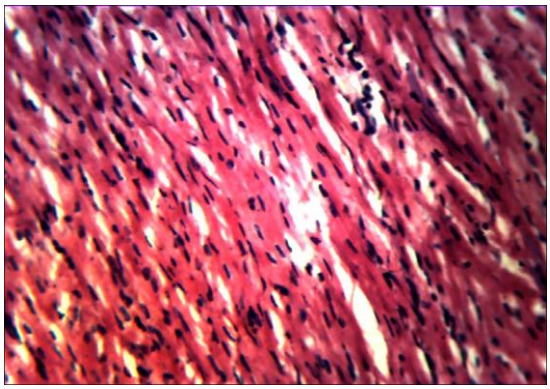
**3.** Artemether-lumenfantrine (70mg/kg) treated mice showing congestion, **(C)**

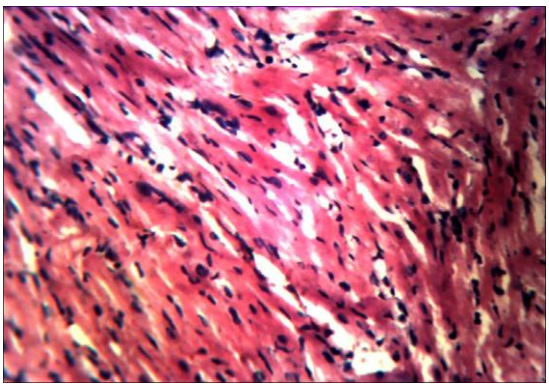
**4.** Promethazine (25mg/kg) treated group showing lymphocye hyperplasia**, (H)**

**5** Mice treated with artemether-lumenfantrine L (70 mg/kg) plus promethazine (25mg/kg) showing slight lymphocyte hyperplasia, **(S)**

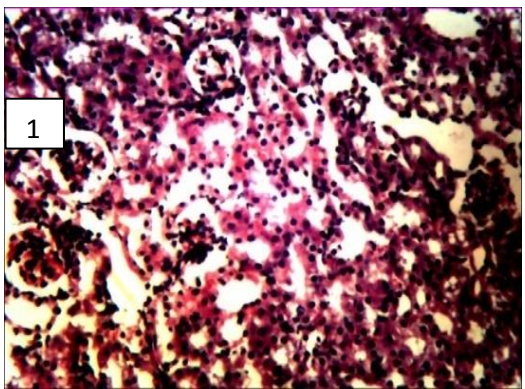


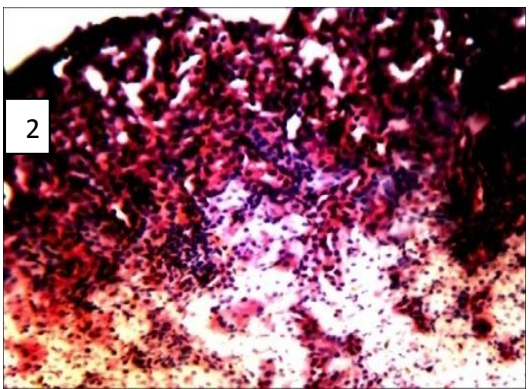


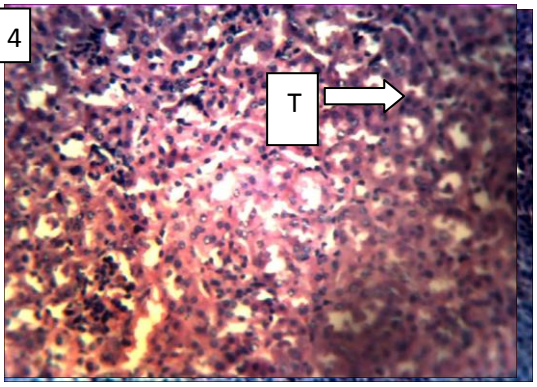


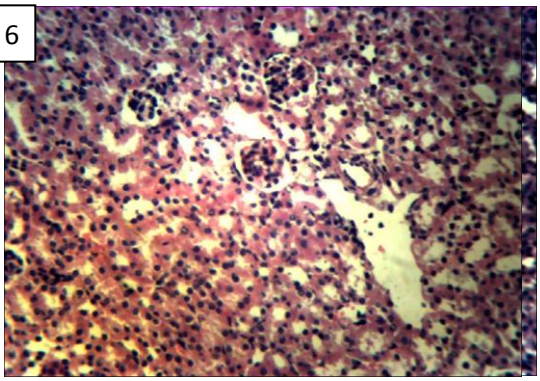


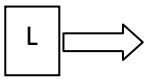
**6.** Artemether-lumenfantrine (70 mg/kg) plus promethazine (50mg/kg) treated group showing moderate lymphocyte hyperplasia, **(M)**





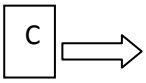




**PLATE III**: Histopathologica ted as follows:(H & E Stain, ×400)

**1.** Normal control mice ormal tubules and glomerulus,**(N)**

**2.** *Plasmodium berghei*in saline showing necrosis and d

**3.** Artemether-lumenfantrine treated group showing slight lymphocyte hyperplasia, (**L**)

**4.** Promethazine (25mg/kg) treated group showing necrosis of tubules and glomerulus, (**T**)

**5.** Artemether-lumenfantrine plus promethazine(25mg/kg) treated group showing lymphocyte hyperplasia, (**L**)

**6.** Artemether-lumenfantrine(70mg/kg) plus promethazine (50mg/kg) treated group showing vascular congestion, (**C**)

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# CHAPTER FIVE

## 5.0 DISCUSSION

World Health Organization (WHO) recommends the use of artemisinin-based combination therapies (ACTs) as first-line treatment in uncomplicated *Plasmodium falciparum* malaria, (WHO, 2010). ACTs therapy is currently regarded as more effective relative to non-artemisinin regimens like chloroquine, and also yielding rapid symptomatic improvement, parasite clearance and a reduction in gametocyte carriage, which could help to reduce malaria transmission (Targett *et al*., 2001; Kokwaro *et al*., 2007; Premji, 2009). Consequent on the use of artemether-lumenfantrine and

promethazine as a treatment for malaria, promethazine is particularly usedfor nausea and vomiting. Artemisinins and promethazineare usually prescribed when malaria strikes, like other therapeutic agents, they are not devoid of unwanted, adverse and toxic effects which are dose dependent.

In this study, promethazine was co-administered with artemether-lumenfantrine to P.berghei infected mice, butpromethazine (25 mg/kg) produced no significant effect on themean parasitaemia level compared to the infected control group. This is consistent with observations from earlier study (Olalubi *el at*.,2011). Artemether-lumenfantrine produced a significant decrease ($p<0.05$) in the mean parasitaemia level. This is consistent with earlier report( Ezike *et al.*,2013). This therefore confirms the antimalarial potentials of artemether-lumenfantrine. The co-administration of artemether-lumenfantrine with promethazine (25mg/kg) produced an insignificant increase in the average mean parasitaemia inhibition compared to co-administration of artemether-lumenfantrine and promethazine at a dose of (50mg/kg), also when the two combinations were compared to the infected control. This may be explained by the metabolism and protein binding properties of artemether-lumenfantrine and promethazine. These drugs are metabolised by the enzyme Cytochrome P450 system, artemether-lumenfantrine is metabolised by CYP3A4 and to a lesser extent by CYP2A6 amongst other substrates, while promethazine is predominately metabolised by the enzyme CYP2A6 (Guyton.,2010). Lumenfantrine inhibits CYP2A6,therefore there is a potential pharmacokinetic interaction of artemether-lumenfantrine with promethazine,(Norvatis, 2009). The co-administration of artemether-lumenfantrine and promethazine may increase the plasma concentrations of promethazine, which has no significant effect on the average percentage parasitaemia inhibition (no potential

antimalaria effects) andresult to quick metabolism of artemether-lumenfantrine in the system. The increased plasma concentration of promethazine on co-administration with artemether-lumenfantrine can be explained by the fact that artemether-lumenfantrine has a higher plasma protein binding than promethazine (Law *et al.,* 2014), therefore promethazine will be unbound, free in plasma, giving a lower average percentage inhibition. The promethazine 25mg/kg dose gives a higher parasitaemia inhibition than the 50mg/kg dose(Ezike *et al.*,2013; Guyton, 2010).

Evaluation of haematological parameters of experimental animals is important in order to help in determining any deleterious effects of foreign compound (e.g drugs) on the blood of animals. Results obtained from this study, revealed that promethazine (25 mg/kg) had a significant effect on blood parameters when compared to artemether-lumenfantrine, but no effects was observed when compared to the infected control. The Hb and PCV were significantly decreased ($p<0.05$) 5 days following administration of the promethazine (25 mg/kg). This may be due to the fact that malaria is known to cause a decrease in Hb and PCV of the blood cells. In addition, promethazine also is known to cause anaemia as a side effect (Rxlist, 2015). Therefore it is not surprising that the Hb and PCV values were significantly decreased ($p< 0.05$).The RBC count though it was decreased, but not statistically significant when compared to artemether-lumenfantrine administered group

Promethazine also significantly ($p \leq 0.05$) reduced the Hb of the infected mice compared to all other treatment groups.This is similar to results from other studies. (Olalubi *et al.* 2011; Sowunmi *et al*., 1998) where Hb values are reduced by promethazine. This could be explained by the fact that promethazine has anaemia as a side effect and this reduces Hb values. Increase in the dose of promethazine will most

likely give more reduction in Hb and this explains why 50 mg/kg of promethazine produced a further lower value of Hb. However, the total variablility between the 25 mg/kg and 50 mg/kg promethazine administered groups is insignificant ($p< 0.05$) when compared to artemether-lumenfantrine alone.

The reduction in the red blood cell (RBC) in promethazine administered and infected control groups compared to the normal control could be attributed to the effects of plasmodium parasite on the RBC (Guyton, 2010). It may also be due to theanaemic effect of promethazine on the RBCs. The low RBC values in the promethazine and infected normal group also indicate an alteration (reduction) in the incorporation of haemoglobin into RBC resulting in the change of morphology and osmotic fragility of the RBC (Ganong,2003**)**. It should be noted that RBCs can be lysed by drugs and infections (as in promethazine and *P. berghei* infection), an effect that increases the deficiency of the enzyme glucose 6-phosphate dehydrogenase (G6PD), which catalyses the initial step in the oxidation of glucose via the hexose mono-phosphate pathway. This generates non adenosine di-phosphate hydrogenase (NADPH) needed for the maintenance of normal red cell fragility. Severe glucose-6-phosphate dehydrogenase (G6PD) deficiency inhibits the killing of bacteria by granulocytes and predisposes the individual to severe infections(Edagha *et al*., 2014). The results of this study is similar to earlier reports on promethazine and amodiaquine, ( Olalubi *et al.,* 2011; Sowunmi *et al.,*1998).

WBCs are the mobile units of the body's protective system, usually transported to sites of serious infection and inflammation. Neutrophils significantly increased ($p< 0.05$) in the infected control and promethazine(25 mg/kg or 50 mg/kg) treated groups compared to the other groups. The most important function of neutrophils is phagocytosis (cellular engulfing of an offending agent-including parasites or drugs) by the process of

opsonisation (Guyton, 2010), which may validate the presence of these agents (neutrophils) in the infected control and promethazine treated groups compared to normal control group.

The presence of lymphocytosis (increase in lymphocyte count) and neutropenia (decrease in neutrophils) in artemether-lumenfantrine treated group suggests that, artemether-lumenfantrine, may stimulate inflammatory response and this is consistent with earlier reported clinical observations, (Ezike,*et al*, 2013).

Platelets were released into circulations after the tail cut for the collection of blood drops for a smear to perform parasite count, to prevent further bleeding from the lateral tail vein of the mice. The platelet indices were decreased in the normal infected control group compared to other groups. When platelet is low, clot retraction is deficient and there is poor constriction of ruptured vessels, resulting in a clinical syndrome: thrombocytopenic purpura, characterised by easy brusability and multiple subcutaneous haemorrhages(Ganong,2003). The platelet indices in Promethazine (25mg/kg) treated group could be seen to be the least, this can be attributed to the fact that promethazine also reduces platelets counts and an increase in the dose to 50mg/kg from 25mg/kg caused a further reduction in the platelet indices.

There was no significant difference in the body weights of the mice across the different treatment groups.

The changes in the liver enzyme system have been used clinically in evaluating the toxicity of any extraneous substance to the living system. This is so because, any derangement of biochemical processes in experimental animals due to the presence of xenobiotics (drug) would produce increase or decrease in the activity of such enzymes including AST, ALT and ALP which are used as indicators of liver injury (Edet, *et al*., 2011). The effects of the different drug treatments on *P. berghei berghei* infected liver

enzymes; the ALT, AST and ALP were evaluated. There was a significant increase, ($p \leq 0.05$) in the ALT, AST and ALP of the promethazine (25 mg/kg or 50 mg/kg) treated group. This is similar to the results obtained from other studies (Edagha *et al*., 2014). This could be due to the increase of liver microsomes (stimulates its production) promethazine is said to have which could lead to an increase in the indices. Also this effect was dose related, the values were higher in the artemether-lumenfantrine + promethazine (50mg/kg) treated group than the artemether-lumenfantrine + promethazine (25 mg/kg) treated group, which is similar to results from other studies (Olalubi, *et al*., 2011 and Sowunmi *et al*., 1998). The artemether-lumenfantrine treated groups produced an insignificant increase, ($p \geq 0.05$) in ALT, ALP and AST compared to the normal infected control group. This result is consistent with earlier reports that artemisinin derivatives cause elevation in serum levels of hepatic enymes (Ezike, *et al,* 2014).

There was no significant difference in the organ-body weight of all the drug treated groups when compared with the normal saline control group, although promethazine treated group as well as infected normal saline conrol group produced a slight increase in the weight of the liver, but it was insignificant ($p<0.05$). This result was not suprising as promethazine was earlier reported to cause increase in liver micrsomes (Nakamura *et al,* 1996),which can lead to an increase in the weight of the liver. However, the statistical insignificance may be due to the duration of use (5 days) or relatively low dose of promethazine (25 mg/kg and 50 mg/kg). Furthermore, the increase in the liver weight of the infected normal saline control group may be due *to P. berghei*infection with no artemether-lumefantrine drug treatment. Malaria or plasmodium infection has been reported in several studies to cause enlargement of the liver (Parnpe*et al.,* 2014). A

similar explanation may be given for kidney, increase in metabolising enzymes as the drugs, promethazine and artemether-lumenfantrine are excreted via the kidney and thus increased action and multiplication of the enzymes.

Generally, in toxicological studies, relative organ body weight changes are often associated with treatment related effects (Seller *et al,* 2007). Liver weight elevation is associated with potent hepatic enzyme-inducing compounds, while changes in kidney weight are linked to renal toxicity, tubular hypertrophy and chronic progressive nephropathy (Sellers*et al,* 2007). The ratio of organ to body weight produced no significant difference with the different treatment groups, although the liver body weight of the promethazine (25 mg/kg) treated group and the promethazine (25 & 50 mg/kg) and artemether-lumenfantrine co-administered group were higher, confirming the potentials of promethazine as a hepatic enzyme inducing compound especially in higher doses.

Drugs produce a wide variety of clinical and pathological hepatic injury; increases (changes) in biochemical markers such as ALT, AST and ALP are indicators of hepatotoxicity. Generally, hepatotoxicity is defined as rise in either ALT level more than three times of upper limit of normal (ULN), AST level more than twice ULN, (Ezike, *et al*.,2014). Since the levels of these biochemical markers of hepatotoxicity are within normal range, the drug combinations may be said to be devoid of hepatotoxicity.

The histopathology of the liver in the treated groups showed varying severity of adaptive responses which consisted of inflammation, hyperplasia, hypertrophy of the hepatocytes with reduced sinusoidal sizes, and pyknotic nuclei, especially in the

parasitized groups. The alteration appeared to be promethazine dose-dependent across the groups. These changes may be indicative of an underlying cellular trauma and morphological change in the tissue cytoarchitecture, a normal reaction of the liver tissue to insults (Kumar *et al.,* 2005).

# CHAPTER SIX

# 6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

## 6.1 SUMMARY

Promethazine produced an insignificant ($p < 0.05$) effect on mean parasitaemia, the average percentage inhibition obtained with promethazine (25 mg/kg) was lower than that of the reference standard drug, artemether-lumenfantrine, which was significantly different at $P< 0.05$. The co-administration of promethazine 25mg/kg and artemether-lumenfantrine gave a higher percentage inhibition than promethazine (50 mg/kg) and

artemether-lumenfantrine, both of which were lower than the reference drug artemether-lumenfantrine.

Promethazine (25mg/kg) alone decreased the Hb, PCV and RBC values, compared to artemether-lumenfantrine alone. But when compared to the infected control, the difference was notstatistically significant ($p > 0.05$). It also reduced the neutrophils and lymphocytes compared to artemether-lumenfantrine. The combination of promethazine (25mg/kg) and artemether-lumenfantrine (10/60mg/kg) gave a reduction in the Hb, PCV and RBC values, compared to artemether-lumenfantrine alone, though the difference was not significant at level 0.05, ($P>0.05$). Promethazine (50mg/kg) in co-administration with artemether-lumenfantrine gave a even lower reduction in the Hb, PCV and RBC, but the difference was also not significant.

The organ weight and organ-body ratio was not significantly different,($P>0.05$) amongst the different treatment groups

The liver enzymes, ALT, AST and ALP, were significantly, ($P <0.05$) increased for promethazine (25mg/kg) compared to artemether-lumenfantrine, infected and uninfected normal saline groups. The co-administration of promethazine (25 mg/kg) and artemether-lumefantrineand co-adminstration of promethazine (50mg/kg) and artemether-lumefantrine were significantly increased, $p \leq 0.05$, compared to artemether-lumenfantrine alone, as well as normal saline infected and uninfected groups.

The histopathological results showed promethazine (25mg/kg) administered alone produced an increased lymphocyte hyperplasia effect on the organs. Promethazine (50mg/kg) co-administered with artemether-lumefantrine and promethazine (25 mg/kg) co-administered with artemether-lumefantrine produced a decrease in lymphocyte hyperplasia compared to promethazine (25 mg/kg) alone.

## 6.2 CONCLUSION

This study established that in the co-administration of promethazine and artemether-lumenfantrine,promethazine did not increase the antimalarial activity of artemether-lumenfantrinebut possesses tendencies of toxicity in prolonged use and increased dose.

## 6.3 RECOMMENDATION

Further investigations be carried out to determine the toxic effects of co-administration of promethazine and artemether-lumenfantrine in prolonged use.

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APPENDIX

**Appendix I: Mean parasitaemia levels by the co-administration of artemether-lumenfantrine and promethazine**

| **Treatment (mg/kg)** | **Day 3** | **Day 5** | **Day 7** | **Day 8** |
|---|---|---|---|---|
| **Normal Saline(0.2ml/kg)** | 5.94±1.416 | 6.09±0.766 | 6.69±0.827 | 6.80±0.678 |
| **A-L (10/60)** | 4.14±0.526 | 3.28±0.678 | 0.64±0.124* | 0.58±0.102* |
| **Pro (25)** | 5.89±1.023 | 5.40±1.577 | 5.65±2.245 | 5.41±0.563 |
| **A-L (10/60) + Pro (25)** | 4.53±1.447 | 4.20±0.522 | 1.36±0.500 | 0.94±0.172 |
| **A-L (10/60) + Pro (50)** | 4.93±0.742 | 4.38±0.380 | 1.94±0.257 | 1.50±0.555 |

N=6, ANOVA, Duncan's post-hoc test, *P≤0.05, AL-Artemether-lumenfantrine, Pro- Promethazine,