STUDY ON THE EFFICACY AND SAFETY OF ARTEMETHER/LUMEFANTRINE IN THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA AMONGST CHILDREN IN KANO.

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DECLARATION

I declare that the work in this thesis entitled, Study on the Efficacy and Safety of Artemether/Lumefantrine in the Treatment of Uncomplicated *Plasmodium falciparum* Malaria among Children under Five Years of Age in the Paediatrics Department of Murtala Muhammad Specialist Hospital, Kano has been performed by me in the Department of Pharmacology and Therapeutics under the supervision of Dr (Mrs.) B.B. Maiha and Dr N.M. Danjuma. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Muhammad Auwal Muhammad _____ OCTOBER, 2014

CERTIFICATION

This thesis entitled STUDY ON THE EFFICACY AND SAFETY OF ARTEMETHER/LUMEFANTRINE IN THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA AMONG CHILDREN UNDER 5 YEARS, IN MURTALA MUHAMMAD SPECIALIST HOSPITAL KANO BY MUHAMMAD AUWAL MUHAMMAD meets the regulations governing the award of the degree of masters of science (Pharmacology) of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Globally, malaria is a major health concern because it causes high morbidity and mortality particularly in children under 5 years because of their low immunity. The development of resistance to chloroquine and sulphodoxine/pyrimethamine in many parts of the world has further worsened the disease. In 2001, WHO recommended the use of artemisinin combination therapy for the treatment of uncomplicated *Plasmodium falciparum* malaria in countries experiencing resistance to chloroquine and other antimalarial monotherapies. In 2005, Nigeria made a shift in the policy of treatment and adopted WHO Artemisinin Combination Therapy policy for the treatment of uncomplicated *Plasmodium falciparum* malaria. The aim of the study was to assess the therapeutic efficacy and safety of the artemether/lumefantrine supplied by the Kano State Government for the treatment of uncomplicated *Plasmodium falciparum* malaria following an observed increase in patients requiring retreatment. It was a prospective evaluation of clinical and parasitological responses to directly observed treatment of uncomplicated *Plasmodium falciparum* malaria using artemether/lumefantrine. Patients of ages 6-59 months numbering 73 who met the inclusion criteria were enrolled into the study after a written informed consent by their parents or guardians. The drug was administered at 0, 8, 12, 24, 48, and 60 hr according to the ages of the patients. They were followed-up on days 0, 1, 2,3,7,14,21, and 28 in which they were assessed clinically and parasitologically. Data obtained were analyzed using the WHO malaria drugs therapeutic efficacy Excel Data Sheet and Graphpad instat software.

The test drug was also subjected to qualitative tests such disintegration test, weight uniformity test, hardness test and friability test to determine the quality of the drug.

The result of the study showed that 63 patients completed the 28 days follow-up period while10 patients were lost to follow-up. There was no early treatment failure observed. However, there was 1 late clinical failure, 8 late parasitological failures and 54 adequate clinical and parasitological response. The cure rate was 85.7% (PCR uncorrected) and failure rate was 14.3% on day 28. The fever clearance time was found to be 40.22 hrs while the parasite clearance time was 52.14 hrs. There was no serious adverse drug reactions observed or reported during the course of the study. The results of the qualitative tests using B.P. 2009 standards were all within the normal limit with a disintegration time of 4.5min, average weight of 244.5 mg ± 1.1 hardness of 9.05 Kg F and the friability was 0.4%. When the above efficacy results are compared with previous efficacy studies conducted in 2002, 2004 and more recently in 2009 it could be seen that the efficacy of artemether/lumefantrine is gradually diminishing with time though it is still within the acceptable limit.

The study showed that the standard six dose regime in malaria treatment with the brand of artemether/lumefantrine was efficacious, safe and qualitative in the treatment of uncomplicated *Plasmodium falciparum* malaria in children less than 5 years of age with an efficacy of 85.7% PCR uncorrected in the 28 days per protocol analysis.

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ACTs	Artemisinin-Based Combination Therapy
ACPR	Adequate Clinical And Parasitological Response
ADRs	Adverse Drug Reactions
A/L	Artemether/Lumefantrine
AUC	Area Under Curve
DHA	Dihydro artemisinin
ETF	Early Treatment Failure
GPI	Glycophosphotidyl Inositol
ITT	Intention To Treat
LCF	Late Clinical Failure
LPF	Late Parasitological Failure
MSP	Merozoite Surface Proteins
PfemP1	*Plasmodium falciparum* erythrocytes membrane Proteins
pLDH	Plasmodium Aldolase
RDT	Rapid Diagnostic Test
TNF	Tumour Necrotic Factor
TRAP	Thrombospondin-Related Anonymous Protein

CHAPTER ONE

INTRODUCTION

1.1 Malaria

Malaria is one of the major health problems in the tropics and affect about 3.3billion people globally in 104 countries (WHO, 2012). It is estimated that 207 million cases of malaria occurred in 2012 globally and 90% of the cases were reported in the African continent (WHO, 2013). The number of deaths due to malaria globally was estimated to be 627,000 in 2012, 90% of this cases were reported in the African continent (WHO, 2013). Nigeria, the Democratic Republic of Congo (DRC) and India accounts for 40% of all estimated cases, while Nigeria and the Democratic Republic of Congo (DRC) account for 40% of estimated total malaria deaths globally (WHO, 2012). In 2012, malaria killed an estimated 482,000 children worldwide, i.e. 1300 children died every day or one child almost every minute. In Nigeria, it is estimated that more than 300,000 children aged less than 5 years die annually from malaria annually (WHO, 2010).

The main cause of malaria in Nigeria is *Plasmodium falciparum,* accounting for 98% malaria cases (FMOH, 2005). Uncomplicated malaria occurs in the majority of those affected, and is the form of illness which presents with symptoms such as fever, headache, muscle pain, anaemia, enlarged spleen, vomiting and sometimes diarrhoea. In children, there could also be rapid breathing and convulsion. Uncomplicated Falciparum malaria does not involve prostration, hypoglycaemia haemoglobinuria, jaundice, renal failure, abnormal bleeding, shock, acidosis, severe anemia, impaired consciousness, multiple convulsions and respiratory distress (WHO, 2000). Chloroquine was the drug of choice in the past for the treatment of uncomplicated malaria. However, because of the development of resistance; it has become less effective in

Nigeria (FMOH, 2005). Resistance to chloroquine first emerged in south East Asia and South America (White, 1999). The resistance later spread to Africa and Oceania. This has resulted in increased morbidity and mortality in malaria endemic areas (FMOH, 2005). A similar process of resistance has affected sulphodoxine/pyrimethamine, though it appears to be slower in West Africa. Resistance to sulphodoxine-pyrimethamine is relatively common in south East Asia (WHO, 2001). Table 1.1 shows result of therapeutic efficacy studies conducted in 2002 and 2004. In Nigeria, the result of the 2002 and 2004 drug efficacy studies carried out in the six geo-political zones indicated that chloroquine and sulfadoxine-pyrimethamine were no longer adequate for National First Line Use (FMOH, 2005).

Table 1.1 **Therapeutic efficacy of antimalarial drugs in Nigeria in 2004.**

S/N	ZONE	CHLOROQUINE	SULFADOXINE/ PYRIMETHAMINE	ARTEMETHER/ LUMEFANTRINE	ARTESUNATE/ AMODIAQUINE
1	SE	3.7%	14.9%	100%	100%
2	SS	9.1%	8.5%	87%	82.5%
3	NC	53.2%	82.7%	100%	96%
4	NW	77.3%	94.2%	100%	100%
5	SW	40.9%	75.6%	100%	100%
6	NE	50.8%	64.8%	100%	100%

Source: FMOH, 2005.

SE- South East, SS-South South, NC-North Central, NW-North West, SW-South West, NE-North East.

The WHO guidelines advice policy review when adequate clinical and parasitological response is less than 75% (Total failure ≥ 25%) and adequate clinical response less than 85% (clinical failure ≥ 15%) (WHO, 2003). WHO recommends the use of artemisinin based combination therapy for uncomplicated malaria due to *Plasmodium falciparum* (WHO, 2010). The *in-vivo* response of the *Plasmodium falciparum* to amodiaquine, halofantrine, mefloquine, and other artemisinin derivatives has been shown to have more than 90% cure rate on day14 (FMOH, 2005). In 2005, the new national policy on malaria treatment came on board in Nigeria in which artemether/lumefantrine was introduced for the treatment of malaria. The combinations affords rapid response and higher cure rate when compared to other antimalarial combinations (White, 1999). Combination therapy is believed to slow parasite developing resistance to the drug (Bloland *et al.*, 2000). The combinations are artesunate plus amodiaquine and artemether plus lumefantrine for uncomplicated malaria. In this research, emphasis was placed on the artemether/lumefantrine combination. The fixed dose of artemether/lumefantrine contains artemether 20 mg and lumefantrine 120 mg. Artemether has rapid onset of action and is rapidly eliminated from the body with half-life of 2-3 hr. (Lefevre and Thomsen, 1999). Lumefantrine is removed slowly with elimination half-life of 4-5 days (Ezzet *et al.,* 1998).The rationale for the combination is that artemether initially provides rapid symptomatic relief by lowering the parasite load, while lumefantrine clears any residual parasite. This action is thought to minimize the development of resistance (Shanks, 2006). Artemether/lumefantrine also reduces the gametocytes carriage thereby reducing malaria transmission (Van vugt *et al.*, 1998). In Nigeria,

the six dose regime of the drug combination given over three days is recommended (FMOH, 2005).

Anti-malarial drug efficacy in uncomplicated *Plasmodium falciparum* malaria is assessed parasitologically by enrolling the age group most affected by the clinical disease. For drugs like artemether/lumefantrine which are rapidly eliminated from the body, require a 28 days follow up, but for drugs that are slowly eliminated like mefloquine, up to nine weeks could be required to document all recrudescence, and where possible the drug levels could be monitored (Stepniewska and white, 2006).

1.2 Statement of the Research Problem

Malaria is prevalent in 104 countries of the tropical and semi tropical world, with 35 countries in central Africa bearing the highest burden of cases and death (WHO, 2010). Of the 35 countries that account globally for approximately 98% of malaria deaths, 30 are in sub Saharan Africa, with 4 countries alone accounting for about 50% of death in the continent. These countries are Nigeria, Democratic Republic of Congo. Uganda and Ethiopia (WHO, 2010). With increased efforts in controlling malaria in Africa in recent years, it is reported that a total of 11 countries in Africa showed a reduction in more than 50% in either confirmed cases of malaria or malaria admission (WHO, 2010).

It is estimated that there are over 100 million cases of malaria in Nigeria, with more than 300,000 deaths of children below the age of 5 annually (WHO, 2010). The emergence of resistance to chloroquine and other antimalarials has resulted in increased morbidity and mortality (Zongo *et al,* 2007). This prompted the WHO to recommend the use of artemisinin based combination therapy (ACT) in such areas where resistance has been reported. The new

national drug policy on malaria treatment has approved the use of ACTs in Nigeria as first line drugs (WHO, 2012).

The Kano state government in its efforts to eradicate malaria in its populace provides free antimalarial drugs to all its patients through the Free Antimalarial Programme. Among the antimalarial drugs provided free are artemether/lumefantrine. Artesunate/amodiaquine and sulfadoxine/pyrimethamine. This has resulted in high patient turnout in the hospitals.

1.3 Justification of the Study

Malaria is a major health problem in Nigeria, where it accounts for more cases of deaths than any other country in the world. WHO estimates that there are 100 million cases with over 300,000 deaths per year in Nigeria (WHO, 2012).

Antimalarial drug resistance results in increase morbidity and mortality, and increase cost to the community (FMOH, 2005). These consequences need to be urgently addressed in Nigeria. Artemisinin combinations were found to be highly efficacious and suitable in the treatment of *P falciparum* malaria (FMOH, 2005).

The high morbidity and mortality rates of malaria in Nigeria makes it important to constantly assess the efficacy of the antimalarials used as first line drugs to be able to eliminate the disease completely (WHO, 2010).

However, the Paediatrics Pharmacy Unit of the department of Murtala Muhammad Specialist Hospital has witnessed documented malaria cases that have been treated over and over again apparently without responding to treatment. To our knowledge, no study of this nature has been

conducted to examine the clinical outcome of treatment of uncomplicated malaria in our environment using these drugs supplied by the Kano State Government.

The study may inform policy formulators about the efficacy, safety and quality of the artemether /lumefantrine supplied to our hospitals for the treatment of uncomplicated *P. falciparum* malaria in the State.

1.4 Aim and Objectives of the Study

The study aims to assess the therapeutic efficacy, safety and quality of artemether /lumefantrine supplied by the Kano State Government for the treatment of uncomplicated malaria due to *P. falciparum* in children less than five years of age in Muratala Muhammad Specialist Hospital, Kano.

The specific objectives of the study are:

- To determine the clinical efficacy of artemether /lumefantrine in patients aged less than five years suffering from uncomplicated *P falciparum* malaria by physical examination for signs and symptoms of malaria during and after treatment with the drug.
- To determine the parasitological efficacy of artemether/lumefantrine in patients aged less than 5 years with uncomplicated *P. falciparum* malaria.
- To evaluate the incidence of adverse events occurring during this treatment.
- To evaluate the quality of the artemether /lumefantrine used by qualitative tests.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria

2.1.1 Historical perspective

Malaria is an infectious disease caused by the plasmodium Species. Five Plasmodia species are known to cause malaria in humans. These are *P. vivax, P. ovale, P. falciparum, P. malariae* and *P. knowles*.

Malaria Parasite is transmitted by the female anopheles mosquito. The disease can be treated in three days but yet can be fatal if diagnosis and treatment are delayed. It is re-emerging as the number one infectious killer and number one priority tropical disease of the World Health Organization. Malaria is the fifth cause of death from infectious disease globally after respiratory tract infection, HIV/AIDS, diarrhoeal disease and tuberculosis. It is the second cause of death in Africa after HIV/AIDS.

Malaria has played a major role in the history of mankind. It continues to cause serious problems in millions of people especially in the poorest parts of the world. Chinese and Egyptian

manuscripts written several thousand years ago in cuneiform on clay tablets, attributes malaria to Nergal, the Babylonian God of destruction and pestilence.

The relationship between malaria and swamps was known even in antiquity and the evil spirit or malaria gods were known to live within the marshes. The Chinese Nei Ching dated 4,700 years ago apparently refers to malaria as repeated attacks of fever associated with enlarged spleen and tendency to epidemic occurrence suggesting *P. vivax* and *P. falciparum.* Infection.

The Sumerian and Egyptians scriptures of 3,500 to 4,000 years ago, refers to fever and enlarged spleen as malaria. The Vedic in the year 3,500 to 2,800 and the Brahmanic scriptures of 2,800 to 1,900 of Northern India contains many references of fever associated with malaria. They make references to autumnal fever as the" king of diseases." The Atharva Veda describes that the fevers were particularly common after excessive rains or when there is too much overgrown grass cover. In 1,800 BC, Dhanvantari said, "Their bite is as painful as that of the serpents and causes diseases". The wound is red as if burnt with caustic or fire accompanied by fever, pain in the limbs, hairstanding, groin pains, vomiting, diarrhoea, thirst, heat, giddiness, yawning, shivering, hiccups, burning, sensation and intense cold.

Malaria appears in the writing of the Greek from 500 BC. Hippocrates, the father of medicine and probably the first malarialogist described the various malaria fever of mans by 400 BC. The Hippocratic corpus was the first document to mention about the splenic changes in malaria and attributes malaria to ingestion of stagnant water. Those who drink stagnant water always have large stiff spleen and hard thin hot stomach, while their shoulder collar bones and faces are emaciated. The fact is that their flesh dissolves to feed the spleen.

Williams Shakespeare in the year 1564-1616 in the Tempest described malaria as ," All the infections that the sun sucks up from bogs, fens, flats on prosper fall and make him by inch meal a disease.

2.1.2 Global spread of malaria

Man and malaria seem to have evolved together. It is believed that human malaria may have had their origin in West Africa and Central Africa on the basis of the presence of homozygous alleles of hemoglobin C and Red Blood Cells duffy negativity that confer protection against *P. falciparum* and *P. vivax* respectively.

Molecular studies recently have shown that human malaria parasites probably jumped on to humans from the apes through the bites of vector mosquitoes. Molecular genetics studies suggest that the pre historic ancestors for malaria parasite was a chloroplast containing free living protozoa which became adapted to live in the gut of a group of aquatic invertebrates. This single celled organism had obligate asexual reproduction within the mid gut lumen of a host species. In the early stage of their evolution, these pre malaria parasites acquired on asexual intracellular form of reproduction is called schizonts. The parasite increases their proliferative potential with this process of schizogony. One of the invertebrates the malaria parasite becomes adaptive with were the aquatic insect larvae, including those of early dipterans. These insects first appeared 150-200 million years ago. Following this period, certain line of ancestral malaria parasites achieved two host cycles which were adapted to the blood feeding habit of the insect host.

The malaria parasite of humans evolved on this line with alternate cycles between humans and their blood feeding female anopheles mosquito host.

Malaria spread across the globe from its origin of West Africa and Central Africa to become the world worst killer disease. The malaria parasite spread to other areas through the journey of man migrating to the Mediterranean, Mesopotamia, the Indian peninsular and South East Asias.

While *P. Malariae* and *P. Vivax* had achieved their widest global distribution, today *P. Malariae* has lost its predominance and *P. vivax* and *P. falciparum* are the most encountered malaria parasites.

P. malariae causes sporadic infection in Africa, parts of India, Western pacific and South America. It is believed that *P. falciparum* had reached India by around 3, 000 years ago. It is also believed that malaria reached the shores of Mediterranean Sea between 2,500 and 2,000 years ago. It reached Northern Europe between 1, 000 to 500 years ago. The series of invasions that swept across the continents helped the cause of malaria parasite as well.

A royal decree was passed in the 11th century Valencia sentencing any farmer to death who planted rice too close to villages and towns. The conflict between rice growers and the authorities continued for centuries. The disease continued to spread with increased spread of rice farming.

By the beginning of the Christian era, malaria was widespread around the shores of Mediterranean, in Southern Europe, across the Arabian Peninsula and in Central, South and South East Asia, China, Manchura, Korea and Japan. Malaria begins to spread into Northern Europe in the dark and middle ages via France and Britain. The growth in international trade in the sixteen century contributed to the spread of the disease, as international trade introduced new source of infections. Europeans and West Africans introduced malaria in the new world at the end of the fifteen century AD. *P. vivax* and *P. malariae* were brought to the new world from

south East Asia by early Trans –pacific voyagers. *P. falciparum* probably reached the Americas through Africans slaves brought by the Spanish Colonizers of Central America.

Initially, the Caribbean and parts of Central and South America were affected and from the mid-18th Century, it spread across the Northern American continent. By the 19th century, malaria reached its global limits with over one and half of the world's population at significant risk.

By mid-20th century, the mortality started dropping as a result of spontaneous decline in contact between human and vectors population as a result of improved living conditions as well as vector control measures. By early 1950's, malaria almost disappeared from North America and from almost all of the European countries. However it became endemic in the tropics.

2.1.3 Transmission of malaria

Malaria is transmitted from man by the female anopheles mosquitoes. Many species of the anopheles vector have been found in various part of the world. There are over 480 species of Anopheles mosquitoes known but only 50 species transmit malaria An. *gambiae* in Africa, An. *freeborni* in North America are the chief vectors in these areas. The habitats of most of the anopheles mosquitoes have been characterized as anthropophilic, endophagic and nocturnal with peak biting at midnight. The blood meal from a vertebrate host is essential for the female mosquitoes to nourish their eggs. The mosquito find their host by seeking visual, thermal and olfactory stimuli and of these, carbon dioxide ,lactic acid, skin temperature and moisture are important source of attraction to mosquito. (Fraden, 1998). When a mosquito bites an infected individual, it sucks the gametocytes, the asexual form of the parasite along with blood. These gametocytes continue the sexual phase of the cycle within the mosquito gut and the sporozoites that develop and then fill the salivary glands of the infested mosquito. When this female

mosquito bites another man for a blood meal, the sporozoites are inoculated into the blood stream of the fresh victim thus spreading the infection. The eggs take about one to two days to develop into adult. The average life span of mosquito is about 2-3 weeks. It can be longer in ideal living conditions. Anopheles mosquitoes breed in natural water collection. The breeding increases in the raining season with wells, ponds, water tanker, paddy fields, etc. acts as breeding ground.

2.1.4. Life cycle of malaria parasite

The malaria parasite has a complex life cycle occurring between the vector mosquito and the vertebrate host. The survival and development of the parasite within the invertebrate and the vertebrate host in intracellular and extracellular environment is made possible by a toolkit of many genes and their proteins that helps the parasite to invade and grow within the multiple cells type and to evade host immune response (Laurence *et al.,* 2002).

The parasite passes through several stages of development such as the sporozoites, merozoites, trophozoites and gametocytes. All these stages have their unique shapes structures and protein complements. The surface proteins and metabolic pathways keep on changing during these different stages that helps the parasite to evade the host immune response, while presenting difficulties for the development of drugs and vaccines.

Sporogony: The asexual phase of malaria parasite life cycle is called sporogony. This phase result in the development of countless infecting forms of the parasite within the mosquito that induces the disease in human host following their infection with the mosquito bite.

When the female anopheles mosquito draws the blood meal from an individual infected with malaria, the male and female gametocytes of the parasite find their way into the gut of the

mosquito. The molecular and cellular changes in the gametes helps the parasite to quickly adjust to the insect host from the warm blooded human host and then to initiate the sporogenic cycle. The male and female gametes fuse in the mosquito gut to form zygotes which develop into ookinetes that burrows into the mosquito midgut wall to develop into oocytes. Growth and development of each oocytes produces thousands of active haploid forms called sporozoites. After the sporogenic phase of 8-15days the

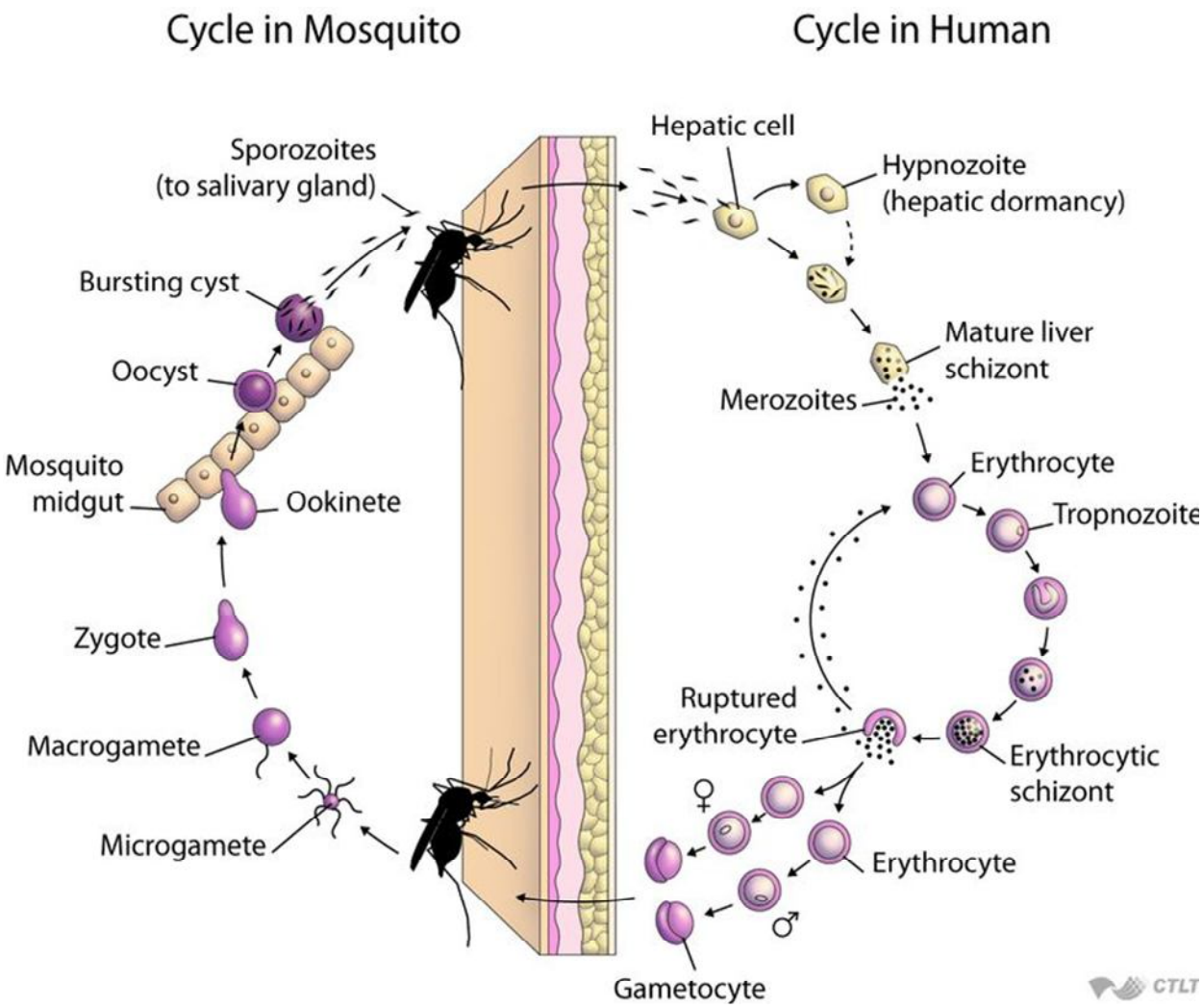

Figure 2.1 Life Cycle of Malaria Parasite

Source: Adopted from Malaria site: http//:ocw.jhsph.edu

oocyte burst and releases sporozoites into the body cavity of the mosquito, from where they travel and invade the mosquito salivary glands. The life cycle of malaria parasite is showed in figure 2.1 above.

When mosquito loaded with sporozoites takes another blood meal. The sporozoites get injected from the salivary gland into human blood stream causing malaria infection in human host (Heather *et al*., 2004).

Schizogony in human host: Man is the intermediate host of malaria where the asexual phase of the life cycle occurs. The sporozoites inoculated by the infested mosquito initiate this phase of the cycle from the liver and the later continue within the red blood cells, which result in the various clinical manifestation of the disease. Following the deposition of sporozoites in the skin, some of the sporozoites are destroyed by the local macrophages while some enter the lymphatics and blood vessels. The sporozoites that enter a lymphatics vessel reach the draining lymph node, where some of the sporozoites partially develop into exo-erythrocytic stage (Ashley *et al.,* 2008).

Sporozoite that enter a blood vessel reach the liver within few hours. It then passes through the liver sinusoids, migrates into a few hepatocytes, multiplies and grows within parasitophorous vacuoles. Each sporozoite develop into a schizont containing many merozoites.The growth and development of the parasite in the liver cells is facilitated by a favorable environment created by the circumsporozoite protein of the parasite. (Miguel *et al*, 2006).

The entire pre-erythrocytic phase last about 5-16 days depending on the parasite species. The merozoites that develop within the hepatocytes are contained inside host cell derived vesicles called merosomes that exit the liver intact thereby protecting the merozoites from phagocytosis by buffer cells. The merozoites are eventually released into the blood stream at the lung capillaries and initiate the blood stage of the infection.

Erythrocytic Schizogony: RBCs are the center stage for the asexual development of malaria parasite. A repeated cycle of parasite development occurs within the RBC. At the end of each cycle, hundreds of fresh daughter parasites are released which invade more RBCs.

The merozoites released from the liver attaches to multiple receptor ligands. This disappearance from circulation into red cells minimizes the exposure of the antigen on the surface of the parasite; thereby protecting these parasites from the host immune response (Olivier *et al.,* 2008).

The invasion of the merozoites into the RBC is facilitated by molecular interactions between distinct ligands on the merozoites and host receptors on the erythrocytes membrane. *P.* vivax invades only Duffy blood group using the Duffy binding proteins and the reticulocytes homology protein found mostly on the reticulocytes.

The more virulent *P. falciparum* uses several receptor families and alternate invasion pathways that are highly redundant. Variety of Duffy binding like homologous protein and reticulocytes binding like homologous protein of P *falciparum* recognizes different RBC receptors other than the Duffy blood group or the reticulocytes receptors. Such sredundancy is helped by the fact that *P. falciparum* has four Duffy binding like erythrocyte binding protein genes in comparism to only one gene in the DBL-EBP family as in the case of *P. vivax* allowing *P. falciparum* to invade any red cell (David *et al*., 2002).

The process of attachment, invasion and establishment of the merozoites into the red cell is made possible by the specialized apical secretory organelles of the merozoites called micronemes, rhoptries and dense granules. The initial interaction between the parasite and the red cells stimulates a rapid wave of deformation across the red cells membrane leading to the formation of a stable parasite- host cell junction. Then the parasite pushes its way through the erythrocyte

bilayer with the help of the actin-myosin motor proteins of the thrombospondin-related anonymous protein family (TRAP) and aldose and creates a parasitophorous cycle vacuole to seal itself from the host –cell cytoplasm, thus creating a hospitable environment for its development within the red cells.

Within the red cells, the parasite number increase rapidly within a sustained cycling of the parasite population. Even though the red cells provide some immunological advantage to the growing parasite, the lack of standard biosynthetic pathways and intracellular organelles in the red cells tend to create obstacle for the fast growing intracellular parasites. These impediments are overcome by the growing ring stages by several mechanisms: by restriction of the nutrient to the abundant haemoglobin, by drastic expansion of the surface area through the formation of a tubovesicular network sand by export of a range of remodeling and virulence factors into the red cells (Olivier *et al*., 2008).

Hemoglobin from the red cells, the main nutrient for the growing parasite is ingested into a food vacuole and degraded. The amino acids thus made available are utilized for protein biosynthesis and the remaining toxic heme is detoxified by heme polymerase and sequestrated as hemozoin. The parasite depends on anaerobic glycolysis of energy, utilizing enzymes such as pLDH, plasmodium aldolase etc. As the parasite grows and multiplies within the red cells, membrane permeability and cystolic composition of the host cell is modified (virgilio *et al*., 2003).

These new permeation pathways induced by the parasite in the host cell membrane help not only in the uptake of solutes from the extracellular medium but also in the disposal of metabolic wastes and in the origin and maintenance of electrochemical gradient. At the same time, the permeation haemolysis of the highly permeabilized infected red cells is prevented by the

excessive ingestion, digestion, and detoxification of the host cell hemoglobin and it's discharged out of the infected Red Blood Cells through the new permeation pathways thereby preserving the osmotic stability of the infected Red Blood Cells (Kiaran *et al.,* 2001).

The erythrocytic cycle occurs every 24 hrs. in the case of *P. knowlesi*, 48hrs in case of *P falciparum*, *P. vivax* and *P. ovale* and 72 hrs in case of *P. malariae*. During each cycle, each merozoite grows and divide within the vacuole into 8-32 fresh merozoites through the stage of ring, trophozoites and schizonts. At the end of the cycle, the infected RBCs ruptures releasing the new merozoites that in turn infect more RBC with sun bridled growth, the parasite numbers can rise rapidly to levels as high as 10^3 per host cells.

2.1.5. Pathogenesis of acute uncomplicated malaria

The manifestations of malaria illness are caused by the infection of the red blood cells by the asexual form of the malaria parasite. This involvement of the red blood cells makes malaria a multi system disease as every organ of the body is reached by blood .(Brian *et al.,* 2008). All types of malaria manifest with common symptoms such as fever, some patient may progress into severe malaria. Although severe malaria is often seen in *P. falciparum* infection, complications and even death have been reported in non-*falciparum* malaria.

At the completion of the schizogony within the red cells, each cycle lasting 24-72 hrs depending on the species of the infecting parasite, newly developed merozoites are released by lysis of infected erythrocytes and along with them, numerous known and unknown substances such as red cells membrane products, hemozoin pigments, and other toxic factors such as glycosylphosphotidylinosit (GPI) are also released into the blood. These products especially GPI activate macrophages and endothelial cells to secrete cytokines and inflammatory mediators such

as tumour necrosis factor, interferon-γ, IL-1, IL-6, IL-8, macrophage colony stimulating factor and lymphotoxin as well as superoxide and nitric oxide. Many studies have implicated the GPI tail common to several merozoite surface proteins (MSP) such as MSP-1,MSP-2, and MSP-4 as a key parasite toxin (Gordon *et al.,* 2007).The systemic manifestation of malaria such as headache, fever, rigors, nausea , vomiting, diarrhoea, anorexia, tidiness, aching joints muscles thrombocytopenia, immunosuppression, coagulopathy, and central nervous system manifestations have been largely attributed to the various cytokines released in response to these parasites and red cells membrane products (Ian *et al*., 2006). In addition to these factors, the plasmodial DNA is also proinflamatory and can induce cytokinemia and fever. The plasmodial DNA is presented by haemozoin which interact intracellularly with the toll- like receptor-9, leading to the release of pro-inflammatory cytokines that in turn induce cyclo oxygenase-2 up regulating prostaglandins leading to the inductin of fever. Hemozoin also have links to the induction of apoptosis in developing erythroid cells in the bone marrow thereby causing anaemia (Lamikanra *et al*., 2009).

2.1.6. Pathogenesis of severe malaria.

The attack of the red blood cells by malaria parasite especially *P. falciparum* leads to structural, biochemical and mechanical modifications of the red cells that can lead to life threatening complications of malaria. While majority of severe malaria mortality are caused by *P. falciparum* infection, complications can occur in non-falciparum as well. Several cases of malaria infections and even death have been reported as a result of infection with *P. vivax* and *P. knowlesi* (Cyrus *et al.,* 2009). Several pathophysiological factors such as the parasite biomass, malaria toxins, and inflammatory response, cytoadherence, resetting and sequestration, altered deformability and fragility of parasitized erythrocytes, endothelial activation, dysfunction and

injury, and altered thrombostasis have been found to be involved in the development of severe malaria. All these phenomena are more profound and wide spread in *P. falciparum* infection compared to non-falciparum infection. Except for severe anaemia, complications such as cerebral malaria, hypoglycaemia, metabolic acidosis, renal failure, respiratory distress are commonly seen in *P. falciparum* infections (Louis *et al.,* 2002).

The cytokines of the proinfalmmatory cascade like tumour necrosis factor, interleukins, interferon-γ, and nitric oxide have both advantages and disadvantages in the pathogenesis of malaria. Cytokines act as haemostatic agents and an early proinflammatory cytokine response help in limiting the infection with the cytokines inhibiting the growth of malarial parasites in lower concentrations. On the other hand failure to down regulate this inflammatory response results in progressive immune pathology, leading to complications. Excessive levels of production; increased cytoadherence that in turn causes microvascular obstruction and more hypoxia, disturbed auto regulation of local blood flow leading to poor circulation and further tissue hypoxia, dyserythropoises, poor red cell deformability and multifactoral anaemia; reduced gluconeogenesis and hypoglycaemia, myocardial depression and cardiac insufficiency, loss of endothelial integrity and vascular damage in the lungs and brain; selective up regulation of vascular and intracellular adhesion molecules; particularly in the brain and placenta, leading to cerebral malaria and placental dysfunction; and activation of leukocytes and platelets, promoting procoagulant activity (Fakhreidin *et al.,* 2003).

Some of the complication seen in *P. vivax* malaria may be related to cytokine mediated injury. *P. vivax* have been reported to induce a greater inflammatory response than *P. falciparum* resulting in higher cytokine release. The pyrogenic threshold is also lower P. *vivax* infections resulting in fever at lower level of parasitaemia. Structural differences in the *P. vivax*

Glycophosphotidylinositil (GPI) that may make it more pyrogenic and greater concentrations of toll-like receptor-9 stimulating motifs within *P. vivax* hemozoin may be responsible for this greater pyrogenicity (Nicolas *et al.,* 2009). A cholesterol containing lipid that has greater activity than GPI-like phospholipids has also been proposed as a putative malaria toxin unique to *P. vivax* and that may also contribute to the pyrogenicity of *P. vivax* (Nadira *et al.,* 2007).

Structural changes in the infected red blood cells and the resulting increase in their rigidity and adhesiveness are major contributors to the virulence of *P. falciparum* malaria. Due to the increased adhesiveness, the red cells infected with late stages of *P. falciparum* adhere to the capillary and post capillary venular endothelium in the deep microvasculature. The infected red cells also adhere to the uninfected red cells, resulting in the formation of red cells rosettes. Cytoadherence leads to sequestration of the parasites in various organs such as heart, lungs, brain, liver, kidney, intestines, adipose tissues, subcutaneous tissues and placenta. Sequestration of the growing *P. falciparum* parasite in these deeper tissues provides them the microaerophilic venous environment that is better suited for their maturation and the adhesion to endothelium allows them to escape clearance by the spleen and to hide from the immune system. These factors help the falciparum parasites to undergo unbridled multiplication thereby increasing the parasite load to very high numbers. Due to the sequestration of the growing parasites in the deeper vasculature, only the ring stage trophozoites of *P. falciparum* are seen circulating in the peripheral blood, while the more mature trophozoites and scizonts are bound in the deep microvasculature, hence seldom seen on peripheral examination, If the cytoadherence resetting sequestration of infected and uninfected erythrocytes in the vital organs goes on uninhibited, it ultimately blocks blood flow, limits the local oxygen supply, hampers mitochondrial ATP synthesis and stimulates cytokine production. All these factors contribute to the development of

severe malaria. Certain proteins expressed on the surface of the infected red blood cells mediate the adhesion of parasitized RBCs to the endothelium and to the uninfected red cells. The most important of such proteins is the *P. falciparum* erythrocyte membrane protein 1 (PfemP1).This is an antigenically diverse protein family that is expressed on the thousands of knob-like excrescences on the surface of the red cells infected with *P. falciparum* trophozoites and schizonts. The PfemP1 is anchored at the red cell membrane skeleton by the knob-associated histidine rich protein. PfemP appears on the surface of the *P. falciparum* infected red cells about 16 hrs after the invasion and that heralds the cytoadherence. PfemP can bind to several adhesive receptors expressed on the endothelial cells such as thrombospondin, CD36, ICAM-1, vascular cell adhesion molecule-1, platelets,/EC adhesion molecule, CD-31, neural cell adhesion molecule-selection and E-selection, integrin avβ3, globular C1q receptor.

2.1.7. Immunity against malaria

Immunity against malaria can be classified into natural or innate immunity and acquired or adaptive immunity.

Natural or innate immunity:

This is an inherent refractorines of the host that prevent the establishment of the infection or an immediate inhibitory response against the introduction of the parasite. The innate immunity is naturally present in the host and is not dependent on any previous infection. Alteration in the structure of haemoglobin or in certain enzymes have been found to confer protection against either the infection or its severe manifestation. These traits are often found in areas of high transmission. Duffy negativity in red cells protect against *P. vivax* infection. It is found to be widely prevalent in Africa and this may be responsible for the virtual elimination of this parasite

from the continent. Certain thalassemias, homozygote haemoglobin C, haemoglobin E and ovalocytosis carrier status have been reported to confer protection against *P. falciparum* or *P. vivax*. Glucose -6- phosphate dehydrogenase, sickle cell haemoglobin confer protection against severe malaria and related mortality (Richard *et al.,* 2002).

Acute malaria infection also induces immediate non-specific immune response that tends to limit the progression of the disease. The humoral and cellular mechanisms of the nonspecific defense are poorly defined. Primordial natural killer cells, intermediate TCR cells and autoantibody producing B-10 cells have been considered as the prime movers of this response. Acquired or adaptive immunity: Acquired or adaptive immunity against malaria develops after infection and its protective efficacy varies depending on the characteristics of the host, place of stay, number of infections suffered.

Following infection with malaria parasites, a non-immunize individual commonly develops an acute clinical illness with very low levels of parasitaemia and the infection may progress to severe disease and death.

After a couple of more infections, anti-disease immunity develops and causes suppression of clinical symptoms even in the presence of heavy parasitamia and also reduce the risk of severe disease. Frequent and multiple infections also lead to the development of antiparasite immunity resulting in high grade immune responsiveness, low level of parasitamia and an asymptomatic carrier status. The presence of genetically and anti-genically distinct strain of the parasite in a given locality and the occurrence of clonal antigenic variations during the course of an infection force the host to mount an immune response against these different strains and antigenic variants.

Immune response have been documented against the various parasite antigens in pre-erythrocytic (sporozoites), asexual erythrocytic (merozoites) and sexual stages (gametocytes).Malarial infection induces both polyclonal and specific immunoglobulin production predominantly IgM and IgG but also of the other immunoglobulin isotopes.

Antibodies may protect against malaria by a variety of mechanisms. They may inhibit merozoite invasion of erythrocytes and intra-erythrocytic growth or enhanced clearance of the infected erythrocytes from the circulation by binding to their surface thereby preventing sequestration in small blood vessels and promoting elimination by the spleen. Opsonization of the infected erythrocytes significantly increase their susceptibility to phagocytosis, cytotoxicity and parasite inhibition by various effector cells such as neutrophils and monocytes or macrophages. Interaction of opsonized erythrocytes with these effector cells induces release of factors such as TNF which may cause tissue lesion but which are also toxic for the parasite (Peter *et al.,* 2002).

Acquired immunity does not last long. In the absence of re -infection for about six month or one year, the acquired immunity turns ineffective and the individual becomes vulnerable to the full impact of a malarial infection once again. Acquired immunity is also rendered less effective during pregnancy. Immunosuppression in HIV/AIDS also increases the risk of clinical malaria, its complications and death (Laith *et al*., 2006).

2.1.8. Diagnosis of malaria

Malaria can be diagnosed based on clinical and laboratory investigations. Clinical investigation is based on fever or history of fever in the last 24 hrs. and or the presence of anaemia. Clinical signs for acute uncomplicated malaria may include, increase body temperature above 37.5 $^{\circ}$C, enlarged spleen or liver especially in children, headache, muscle pain, anemia, vomiting and

sometimes diarrhea, rapid breathing and convulsion especially in children. Severe malaria may involve prostration, hypoglycemia, haemoglobinuria, jaundice, renal failure, acidosis, impaired consciousness and circulatory collapse. Clinical diagnosis alone can result in over-diagnosis of malaria. Hence parasitological confirmation is strongly recommended (FMOH, 2010).

Parasitological conformation is needed in all suspected cases of malaria. However in areas of high transmission, children under 5 years can be treated on clinical basis where parasitological confirmation is not feasible or in cases of suspected severe malaria (FMOH, 2010).

Prompt and accurate diagnosis is part of effective disease management. High sensitivity of malaria diagnosis is important in identifying positive cases. High specificity is important in identifying negative cases, which can reduce unnecessary treatment with antimalarials and improve differential diagnosis of febrile illness.

There two methods used for parasitological diagnosis; light microscopy and rapid diagnostic test.

Microscopy is the standard method for parasitological diagnosis for malaria parasite. It is done by examining a stained thick or thin blood smear for the presence of malaria parasites. Thick films are recommended for parasite detection and quantification and can be used to monitor response to treatment. Thin films are recommended for species identification. Microscopic examination of stained blood films by a trained microscopist has a sensitivity range of 86-98% with lower sensitivity in detecting parasitaemias lower than 320 cells/µl .Factors such as the stage of the malaria infection and previous medication may reduce parasitaemia below the detectable threshold and calls for repeat examination.

Antimalarial treatment should be limited to test positive cases. The negative cases should be reassessed for other common causes of fever. The benefit of parasitological diagnosis depends on

the health-care adhering to the results in managing the patient. However the severity of the disease justifies the use of antimalarial medicines in test negative cases. The risk of false negative microscopy is higher if the patient has received a recent dose of antimalarial agent.

Malaria rapid diagnostic tests (RDT) detect parasite-specific antigens or enzymes and some have ability to detect species. The RDT signifies the presence of the antigens by colour change on nitrocellulose strip, which is impregnated with malaria antibodies.

The RDT provides simple guide to the presence of clinically significant malaria infection and complements microscopy based diagnosis where such services are not available. RDT should however not replace microscopy.

2.2. Classification of Antimalarial Drugs:

Antimalarial drugs can be classified according to antimalarial activity and according to their chemical structure.

2.2.1. Classification based on antimalarial activity

1) Tissue schizonticides for causal prophylaxis:

These act on the primary tissue forms of the plasmodia which after growth within the liver initiate the erythrocytic stage. By blocking this stage, further development of the infection can be prevented. Pyrimethamine and primaquine act by this mechanism. However, since it is impossible to predict the infection before clinical symptoms begins, this mode of therapy is more theoretical than practical.

11) Tissue schizonticides for preventing relapse

These drugs act on the hypnozoites of *P. vivax* and *P. ovale* in the liver that causes relapse of symptoms on reaction. Primaquine and pyrimethamine are the proto type drugs that through this way.

111) Blood schizonticides

These drugs act on the blood forms of the parasite and thereby terminates clinical attacks of malaria. These are the most important drugs in antimalarial chemotherapy. They include artemether/lumefantrine, chloroquine, quinine, mefloquine, pyrimethamine, sulfadoxine and tetracyclines.

1V) Gametocytocides

These drugs destroy the sexual forms of the parasite in the blood and thereby prevent transmission of the infection to the mosquito. Chloroquine and quinine have gametocidal activity against P *vivax* and P *malariae* but not against *P falciparuim.* Primaquine has gametocidal activity against all plasmodium parasites including *P. falciparuim*.

V) Sporonticides

These drugs prevent the development of oocytes in the mosquito and thus prevent the transmission of the parasite. Primaquine and chloroquine have this activity.

Thus in effect the treatment of malaria would include a blood schizonticide, a gametocidal and tissue schizonticide. A combination of chloroquine and primaquine is thus needed in all cases of malaria.

2.2.2. Classification based on chemical structure

1) Acryl amino alcohols: Quinine, quinidine, mefloquine and halofantrine

11) 4-aminoquinolines: -- Chloroquine, amodiaquine.

111) Folate synthesis inhibitor: Type1- competitive inhibitors of dihydropteroate synthase eg sulphones and sulphonamides.

Type 2- inhibit dihydrofolate reductase e.g. proguanil, pyrimethamine.

1V) 8—aminoquinolines- Primaquine

V) Antimicrobials—Tetracyclines, doxycycline, clindamycin.

V1) Peroxides—Artemisinins-artemether, arteether, artetinic acid

V11) Napthoquinones: Atovaquone

V111) Iron chelating agent: Desferroxamine

2.3. Properties of some Common Antimalarials Drugs

2.3.1. Artemether/lumefantrine

Artemisinin is a sesquiterpene lactone ring which has been used for treating fevers in China for over two millennia. It was identified in1972 as a colourless crystalline substance with molecular weight of 202 Da, molecular formula of C_{15} H_{22} O_5 and melting point of 156-157 ^{O}C.

In 1964, during the Vietnam War, the number of soldiers that died of drug resistant falciparum malaria was much higher than the number of casualties from both combating sides. The then

leadership of Vietnam asked China for help as the malaria parasites were resistant to frequently used antimalarials.

In 1967, the Government of the People's Republic of China began a programme identify the antimalarial principles in plants used in traditional Chinese medicine (Klayman, 1985). In 1971, workers at the Pharmaceutical Institute of Academy of Traditional Chinese. Medicine succeeded in showing that the extract of Qinghao killed *P. berghei* in mice. In 1972, the active principles of Qinghaosu from Qinghao were identified. These active principles are now called artemisinin.

Description

Artemether/lumefantrine tablets contains a fixed combination of two antimalarial active ingredients, artemether, an artemisinin derivative and lumefantrine. Both the drugs are schizonticidal. The chemical name of artemether is (3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR) decahydro-10-methoxy-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano [4, 3,-j]-1, 2-benzodioxepine. Artemether is a white crystalline powder that is freely soluble in acetone, soluble in methanol and ethanol and practically insoluble in water. It has the empirical formula of $C_{16}H_{26}O_5$ with molecular weight of 298.4 .The chemical structure of artemether is shown in figure 2.1 below.

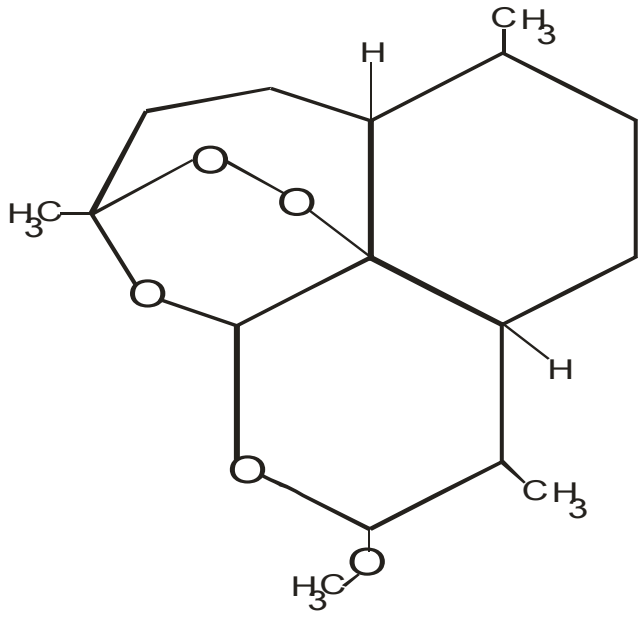

Figure 2.2 The Structure Of Artemether

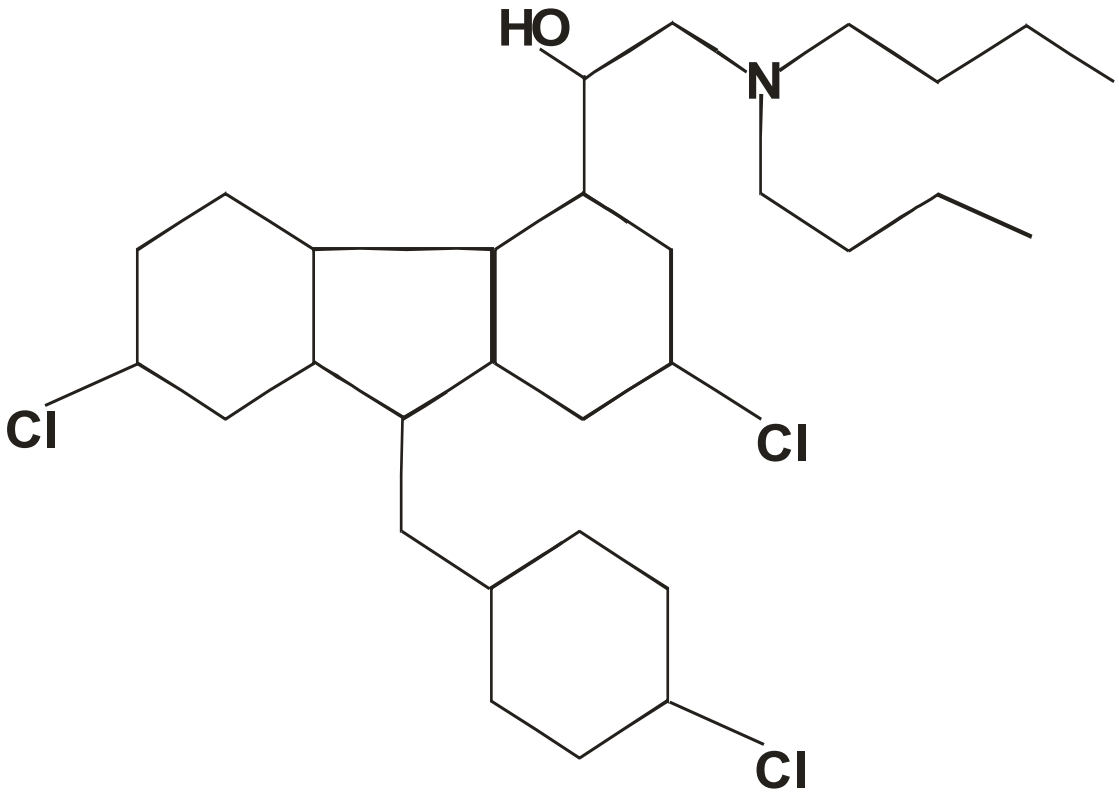

Figure 2.3 The chemical structure of Lumefantrine

The chemical name of lumefantrine is (±)-2-butylamino-1- [2, 7-dicloro-9-(4-clorobenzylidene)-9H-fluorine-4-yl] ethanol.

Lumefantrine is a yellow, crystalline powder that is freely soluble in N, N-dimethylformamide, chloroform and ethyl acetate; soluble in dichloromethane; slightly soluble in ethanol and methanol, insoluble in water. It has the empirical formula of $C_{30}H_{32}C_{l3}NO$ with molecular weight of 528.9 .The chemical structure of lumefantrine is shown in figure 2.2

2.3.1.1 Mode of action:

Artemether/lumefantrine tablets contain a fixed dose combination of artemether/lumefantrine in the ratio of 1: 6.

Artemether containing endoperoxides interacts with haem or ferrous ions in the digestive food vacuole of *P. falciparum* (Meshnick *et al*., 1991). This result in the formation of potentially toxic oxygen and carbon centered radicals. These radicals cause lyses of the digestive food vacuole (Postma *et al*., 1996).

Artemisinis also has the ability to reduce fever by sending of signals to hypothalamus thermo regulatory center. This is as a result of the presence of new previously unknown cyclo oxygenase enzyme COX-3 found in the brain and spinal cord. The COX-3 is selectively inhibited by artemether. It is this inhibition that is responsible for relieving pain and reducing fever produced by malaria.

More recently, an alternative hypothesis has been proposed that it acts by inhibiting *Plasmodium falciparum* encoded sarcoplasmic-endoplasmic reticulum calcium ATPase (Eckstein-Ludwig et

al., 2003). The exact mode of action of lumefantrine is unknown. However, available information suggest that it inhibits the formation of β-haematin by forming a complex with haem and inhibits synthesis of nucleic acid and proteins.

2.3.1.2 Pharmacokinetics.

Absorption: following oral administration of artemether/lumefantrine tablets, artemether is rapidly absorbed with peak plasma concentration being attained within 2 hrs (Mordi *et al*., 1997). Absorption of lumefantrine, a highly lipophilic compound, starts after a lag time of 2 hrs. Peak plasma concentration is achieved within 6 to 8 hrs after oral administration (Karbwang *et al*., 1997). Administration of artemether/lumefantrine with fatty food increases bioavailability of artemether by 2 folds (Ashley *et al*., 2007) and lumefantrine by more than 16 folds.

Distribution:

Artemether and lumefantrine are highly bound to plasma proteins. Artemether is 95.4% bound to plasma proteins (Colussi *et al*., 1999) while lumefantrine is 99.7% bound. Dihydroartemisinin derivative is 47 to 76% bound to plasma proteins.

Biotransformation:

The metabolism of artemether is catalyzed predominantly by CYP 450 3A4 isoenzyme, to dihydroartemisinin, the active metabolite (Van Agtmeal *et al*., 1999). Lumefantrine is also metabolized by CYP 450 3A4 isoenzyme to desbutyl lumefantrine. Lumefantrine also inhibits the activity of CYP 450 2D6 at therapeutic plasma concentration.

Elimination

Artemether and dihydroartemisinin are removed from plasma with elimination half- life of 2hours. Lumefantrine is slowly removed with elimination half- life of 4 to 5 days (Ezzet *et al*., 1998). No urinary excretion data are available for humans.

Demographic characteristics such as sex and weight appear to have no clinically relevant effects on the pharmacokinetics of artemether/lumefantrine.

2.3.1.3 Indications and Usage:

Artemether/Lumefantrine tablets are indicated for the treatment of acute, uncomplicated malaria infection due to *P. falciparum.* The combination has been shown to be effective in geographical regions where resistance to chloroquine has been reported. Artemether/lumefantrine is not used in severe malaria. The dosage of artemether/lumefantrine tablets according to body weight is shown in table 2.1.

Table 2.1 Dosage of Artemether/Lumefantrine Tablet According to Body Weight

BODY WEIGHT	NO OF TABLETS TO BE TAKEN
5 to □15kg	1 tablet twice daily for 3days
15 to25kg	2 tablet twice daily for 3days

25 to35kg	3 tablet twice daily for 3days
35kg and above	4 tablet twice daily for 3days

2.3.1.4 Contraindication:

The combination is contraindicated in patients that are hypersensitive to artemether, lumefantrine or any of the excipients.

Adverse Reaction:

The most common adverse reactions are headache, anorexia, dizziness, asthenia, arthralgia and myalgia. In children there could be pyrexia, cough and vomiting.

2.3.1.5 Drug interaction:

CYP450 3A4 inhibitors: This results in increase in exposure in terms of area under curve (AUC) of artemether, 2.3 folds DHA 1.5 folds and lumefantrine 1.6 folds. The increase in concentration of lumefantrine could lead to QT prolongation. Artemether/lumefantrine should be used cautiously with other drugs that inhibits CYP450 3A4 e.g. antiretroviral drugs, macrolides antibiotics, antidepressant and imidazole antifungals.

Antimalarial**s:** Quinine should be used cautiously with artemether/lumefantrine due to the long elimination half- life of lumefantrine and the potential for additive effects on the QT interval.

2.3.2 Quinine

It is the main alkaloid obtained from the bark of cinchona bark tree which occurs in South America. The Augustinian monk called Calancha first wrote about the curative properties of Cinchona powder in his book “Fevers and Tertians” in 1633. By 1640, the bark has already

reached Europe. Eminent philosopher Cardinal de lugo popularized the bark in Rome hence it is called the cardinal's bark. In 1820, Pelletier and Caventou isolated quinine and cinchonine from the bark of the plant. .Quinine was isolated entirely from the natural source due to the difficulty in synthesizing the complex molecule.

2.3.2.1 Mode of action

Quinine is a blood schizonticide, although it also has gametocidal properties against *P. vivax* and *P. malariae.* It acts by inhibiting haem polymerase, thereby causing the accumulation of its toxic substrate, haem. Quinine is less effective and more toxic than chloroquine. It is however used in treatment of severe malaria in areas known to have resistance to chloroquine.

2.3.2.2 Pharmacokinetics

Quinine is readily absorbed after oral or intramuscular injection. Peak plasma concentrations are achieved with 1-3 hrs after administration with plasma half- life of 11hrs. In acute malaria, the volume of distribution of quinine decreases while clearance is reduced.

2.3.2.3 Adverse effects

Quinine is a highly toxic drug. It could be mild in usual therapeutic dose or could be severe in large doses. Cinchonism which is one of the adverse effects consist of ringing in the ears, headache, nausea and visual disturbances. Impairment of the eighth nerve results in tinnitus, decrease auditory acuity and vertigo. Visual symptoms consist of blurred vision, disturbed colour perception, photophobia, diplopia, night blindness, and rarely even blindness which are due to direct Neurotoxicity.

Gastrointestinal symptoms such as nausea, vomiting, abdominal pain and diarrhea may be observed. Rashes, sweating angioedema can occur. Excitement, confusion, delirium are common in some patients, while coma, respiratory arrest, hypotension and death can occur with over dosage. Quinine can also cause renal failure. Massive haemolysis and haemoglobinuria can occur especially in pregnancy or on repeated use. Hypoprothombinemia and agranulocytosis are also seen.

Quinine has little effect on the heart in therapeutic doses. It reduces the excitability of the motor end plate and therefore antagonizes the effect of physostigmine.

Quinine stimulates insulin secretion and in therapeutic doses can cause hypoglycemia which in malaria can go unnoticed and this can lead to death .It is therefore advisable to monitor blood glucose levels every 4-6 hr. during treatment with quinine especially in severe malaria and in pregnancy. Quinine induced hypoglycemia can occur even after administration of 50% dextrose. It is therefore advisable to use 10% dextrose as maintenance. Resistant hypoglycaemia due to quinine can be treated with ocreotide injection 50mcg subcutaneously every 6-8hrs.

2.3.2.4 Contraindications

Hypersensitivity in the form of skin rashes, angioedema, visual and auditory symptoms require withdrawal of the drug. It is contraindicated in tinnitus and optic neuritis. Quinine should be used with caution in patient with atrial fibrillation. It is also contraindicated in patient suffering from myasthenia gravis and hemolysis.

.Dose: Oral: 10mg/kg every 8hrs for 4days and 5mg/kg every 8hrs for 3 days.

Intravenous: 20mg of salt/kg in 10ml/kg isotonic saline or 5% dextrose every 4hrs, then10mg of salt/kg in saline or dextrose over 4hrs, every 8hrs until patient is able to take oral medication or continue with intravenous medication for 5-7days.

Intramuscular: 20mg/kg salt, followed by 10mg/kg 8 hrly by deep intra muscular injections for 5-7 days.

Availability: It is available as tablet and as capsule of 300 mg and 600 mg of the base. It is available as injection of 300mg/ml.

2.3.3. Chloroquine

It is a prototype antimalarial drug most widely used to treat all types of malarial infections. The drug is the cheapest and safe of all antimalarials presently.

2.3.3.1 Mode of action

The mechanism of action of chloroquine is unclear. As a result of the alkaline nature of the drug it reaches a high concentration within the food vacuole of the parasite and raises the pH. It is found to induce rapid clumping of the pigment. Chloroquine inhibits parasitic enzyme haem polymerase that converts the toxic haem into non-toxic haemazoin, thereby resulting in the accumulation of toxic haem within the parasites. Chloroquine is highly effective against erythrocytic forms of *P. vivax, P. malariae, and P. ovale,* sensitive strains of *P. falciparum* and gametocytes of *P. vivax.* It may interferes with the biosynthesis of nucleic acids. Other mechanisms suggested include formation of drug-haem complex, intercalation of the drug with the parasite deoxynucleic acid.

2.3.3.2 Pharmacokinetics

About 90% of the drug is absorbed from the gastrointestinal tract and rapidly absorbed from intramuscular and subcutaneous sites. Chloroquine has a large volume of distribution due to its extensive sequestration in the liver tissue, spleen, kidney and lungs hence the need for a large dose of the drug.

Therapeutic levels of the drug persist for 6-10days. Elimination half- life is 1-2 months. Half of the drug is excreted unchanged by the kidneys, and the remaining is converted to active metabolites in the liver. It rapidly controls acute attack of malaria with most patients becoming afebrile within 24-48 hrs. It is more effective and safer than quinine for sensitive cases.

2.3.3.3 Adverse effects:

It is relatively safe antimalarial drug however, therapeutic doses it can cause dizziness, headache, diplopia, disturbed visual accommodation, dysphagia, nausea, malaise nd pruritis of palm, soles and scalp. It can cause visual hallucinations, confusion, and occasionally frank psychosis. The side effects do not warrant stoppage of treatment. It can exacerbate epilepsy and when used as prophylactic agent at 300 mg weekly, it can cause retinal toxicity after 3-5 years of treatment Intramuscular injection can cause hypotension and cardiac arrest especially in children.

2.3.3.4 Contraindication:

Chloroquine should be used with caution in patient with hepatic disease, severe gastrointestinal and neurological or blood disorders. The drug should be discontinued in the event of such problem during therapy. It should not be co-administered with gold salt and phenylbutazone, because all the three drugs can cause dermatitis. Chloroquine interferes with antibody response to human diploid cell rabies vaccine.

Availability Chloroquine phosphate tablet 250 mg contains 150 mg of the base. Chloroquine hydrochloride injection contains 40 mg/ml.

Dose 10 mg/kg stat, then three doses of 5 mg/kg over 36-48 hrs.

2.3.4 Mefloquine:

Mefloquine was discovered during the Vietnam War as a result of research into newer antimalarial agents. This was done with the aim of protecting the American soldiers from multi drug resistant falciparum malaria. The drug is therefore reserved for multi-drug resistant *P. falciparum* malaria only.

2.3.4.1 Mode of action:

Mefloquine has been found to produce swelling of the *P. falciparum* food vacuoles. It may act by forming toxic complexes with free haem that damages membranes and interact with other plasmodial component. It is effective against blood forms of *P. falciparum* malaria including chloroquine resistant types.

2.3.4.2 Pharmacokinetics

Mefloquine is available only for oral use because parenteral preparations are known to cause local reaction. The drug is rapidly absorbed and is highly bound to plasma proteins. The elimination half-life is about 2-3 weeks and is excretion is mainly in the faeces.

2.3.4.3 Adverse effects

Mefloquine is well tolerated when given in therapeutic doses of 1500 mg. It can cause nausea, vomiting, abdominal pain and dizziness in doses more than 1000 mg. The drug can sometimes cause nightmares, sleeping disturbances, ataxia, sinus bradycardia, sinus arrhythmia, postural hypotension and acute brain syndrome consisting of fatigue, asthenia, seizures and psychosis.

2.3.4.4 Contraindicatioin

Mefloquine should be used with caution in patient with heart block, patient taking beta blockers, patient with history of epilepsy and psychiatric disease. It should be avoided in the first trimester of pregnancy and pregnancy should be avoided within three months of taking the drug.

Mefloquine should not be used for prophylaxis in pregnancy especially during the first trimester. It is also contraindicated in patient with history of seizures, severe neuropsychiatric disturbances or adverse reactions to quinolone antimalarials like chloroquine and quinine. Mefloquine should not be used concomitantly with these drugs because of increased risk of cardiotoxicity and the risk of convulsions. Mefloquine is said to increase the risk of seizures in patients taking valproate. It may compromise adequate immunization by live typhoid vaccine. Patients taking mefloquine should not operate any machinery or drive automobile

Dose: 15 mg/kg as a single dose. If the dose is more than 1000 mg, the second dose can be given after 4-8 hrs. to minimize gastric irritation. The total dose should not exceed 1500 mg

Availability: It is available as 250 mg tablet.

2.3.5. Primaquine

Primaquine is an essential co-drug given with chloroquine in treating all cases of malaria. It is highly effective in the gametocytes of all plasmodia and therefore prevents the spread of the disease to the mosquito from the patient. It is also effective against the dominant tissue forms of *P. vivax* and *P.* ovale malaria and there by offers radical cure and prevents relapse. It has insignificant activity against the asexual form of the parasite and therefore it is always used in conjunction with a blood shizonticide and never as a single agent.

2.3.5.1 Mode of action:

The mechanism of action is not well understood. It may act by generating reactive oxygen species or by interfering with the electron transport system in the parasite.

2.3.5.2 Pharmacokinetics

Primaquine is well absorbed after oral administration and rapidly metabolized by the liver. Its elimination half-life if about 6 hrs. The metabolites of primaquine have oxidative properties and can cause hemolysis in susceptible patients.

2.3.5.3 Side effects

Primaquine is well tolerated when given in therapeutic doses. It can cause epigastric distress and abdominal cramps. It is therefore advisable to take the drug with meal to minimize this effect. Mild anemia, cyanosis, methaemaglobininemia may also occur. Severe methaemaglobinemia can

occur rarely in patient with deficiency of NADH methemoglobin reductase. Agranulocytosis and granulocytopenia are rare complications.

Patient with deficiency of glucose 6 phosphate dehydrogenase will develop haemolytic anaemia on taking usual dose of primaquine. It should not be taken by patient with severe systemic illness that is likely to cause leucopenia. It should not be used with drugs that cause bone marrow depression.

Availability: Primaquine is available as tablet of 2.5 mg, 7.5 mg, and 15 mg of the salt.

2.3.6. Sulfadoxine/Pyrimethamine

This is a very useful adjunct in the treatment of uncomplicated, chloroquine resistant *P. falciparum* malaria. It is now used in combination with artesunate for the treatment of *P. falciparum* malaria. It is also used in intermittent treatment in pregnancy.

2.3.6.1 Mode of action

Pyrimethamine inhibit dihydrofolate reductase of plasmodia and thereby blocks the biosynthesis of purine and pyrimidines, which are essential for DNA synthesis and cell multiplication. This leads to failure of nuclear division at the time of schizont formation in erythrocytes and the liver.

Sulfadoxine inhibit the utilization of para-amino benzoic acid in the synthesis of dihydropteroic acid. The combination offers two step synergistic blockade of plasmodial division.

2.3.6.2 Pharmacokinetics

Pyrimethamine is slowly and completely absorbed after oral administration. It has an elimination half- life of 80-95 hrs. Suppressive blood levels may be found in the plasma for up to 2 weeks.

Sulfadoxine is rapidly absorbed from the gut and is bound to plasma proteins. It is metabolized in the liver and excreted in the urine. It can cross the placenta freely and is a long acting sulfonamide with an elimination half- life of 7-9 days.

2.3.6.3 Adverse effects

Pyrimethamine can cause occasional skin rashes and depression of hematopoiesis and excessive dose can cause megaloblastic anaemia.

sulphadoxine can cause agranulocytosis, aplastic anaemia, hypersensitivity reactions like fix drug reaction, erythema multiforme of the Steven Johnson type, exfoliative dermatitis, serum sickness, liver dysfunction, anorexia, vomiting and acute hemolytic anaemia.

Sulphadoxine is contraindicated in patients known to have hypersensitivity to sulfonamides, infants below 2 months of age, patients with advance renal disease, and first and last trimester of pregnancy.

Availability Pyrimethamine and sulphodoxine are available as tablet of 25 mg pyrimethamine and 500 mg sulfadoxine respectively.

Dose Children 1-5 yrs. half the tablet is given. .5-9 years a tablet is given and adults are given 3 tablets at once.

2.3.7. Antimicrobials

Antimicrobials are used to treat a wide range of infections including malaria.

2.3.7.1 Tetracyclines

These antibiotics drugs are used to treat a wide range of infections including malaria.

Mode of action

Tetracyclines are bacteriostatic agents that act by inhibiting 30s ribosomal subunit in protein synthesis. They are effective against a wide range of microorganisms both aerobic and anerobic gram positive and gram negative bacteria. They are effective against rickettsia, mycoplasma, ureaplasma, chlamydia, legionella, spirochaetes, brucellas, helicobacter pylori, yersinia and some atypical mycobacteria and plasmodia.

Pharmacokinetics

These group of drugs are incompletely absorbed from the gut after oral administration. The absorption may be hampered by heavy metals iron when taken concomitantly. They are widely distributed in the tissue and accumulate in the liver, spleen, bone marrow etc. Tetracyclines are excreted through the kidneys and they may be hampered by renal failure.

Adverse effects

Include, Gastro intestinal irritation, nausea, vomiting, diarrhea, photosensitivity, hepatotoxicity, aggravation of uremia, staining of the teeth in children.

Use They are useful in the treatment of drug resistant *P. falciparum* malaria. They used in combination with other antimalarial drugs like quinine because they act very slowly.

Contraindication:

They are contraindicated in children below the age of 8 yrs and in pregnant women.

Tetracycline and Doxycycline are commonly used.

Dose: Tetracycline is usually given at the dose of 250 mg every 6 hr. for7- 10 days, while doxycycline is given 100 mg twice daily for 7-10days

2.3.7.2 Doxycycline is used for short term prophylaxis in the dose of 100mg daily against *P. falciparum* malaria.

2.3.7.3 Clindamycin

It act by inhibiting protein synthesis by binding to the 50s ribosomal subunit of ribosomes .It can be used for drug resistant malaria along with quinine at the dose of10mg/kg 8hrly for 5days

Side effects include pseudomembraneous colitis and skin rashes.

2.3.7.4 Fluoroqiunolones

Both ciprofloxacin and norfloxacin have been found to have antimalaria activity both *in- vitro* and *in -vivo*. However results are not consistent.

2.3.7.5 Azithromycin

This drug is also found to have antimalarial activity and has been found to be useful as a causal prophylactic agent. It was found to be effective in doses of 300mg stat, followed by 250mgdaily for 7 days as a prophylactic agent against chloroquine resistant *P. falciparum* malaria.

2.3.8 Atovaquone:

This is a synthetic hydroxynapthoquinone developed in the early 80s. Atovaquinone has been found to be useful against the plasmodia. It is a highly lipophilic molecule that supposedly interferes with mitochondrial electron transport and thereby ATP and pyrimidine biosynthesis It is found to target cytochrome bc_1 Complex and disrupt the membrane potential .It has poor bioavailability after oral administration and may be increased by fatty meal .It has a half-life of 2-3 days and it undergoes enterohepatic circulation . It is available as 750mg tablet.

It can cause skin rashes, vomiting, diarrhea and headache. Safety in pregnancy, lactation, children, and elderly is not yet established.

2.3.9 Pyronaridines:

It structurally resembles amodiaquine and has been found to be highly effective against chloroquine resistant strains in China.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study Site

The study site was Murtala Muhammad Specialist Hospital, Kano. It was carried out in the months of October and November, 2013. This hospital was established in 1938. It is located at Shahuci; along Kofar Mata road in the old city of Kano Municipal Local Government Area of Kano Stat North Western Nigeria. It has a bed capacity of 750 patients with patient attendance of 3,500 per day. The hospital is patronized by people of the ancient city and neighbouring countries such as Niger Republic and Chad. Kano Municipal LGA is inhabited mostly by Hausa/Fulani.

3.2 Study Population

The population consists of children less than 5 years of age with uncomplicated malaria due to *Plasmodium falciparum* attending the facility. The guardians or parents of prospective patients signed a consent form to give permission to their children or wards to participate in the study. The purpose of the study, it benefits and risks was translated in Hausa (Appendix III), the native language which is widely spoken in the area.

3.3 Sample Size

The sample size was determined based on the WHO standard protocol assuming the anticipated population proportion of clinical failure of 20%, with 95% confidence level and 10% precision. Sample size was adjusted to take care of loss to follow- up and withdrawals. This adjustment is usually 20% addition for studies that involve longer follow-up period of 28 days and above as in artemether/lumefantrine (Stepniewska and White, 2006). Table 3.1 shows how the sample size determination was done.

From the table below where P=0.20% d =0.10, a sample size of 73 was used with 20% adjustment,

n= (1+0.20) x61 =73.2, where n is the sample size.

Table 3.1 Sample Size determination

	Anticipated population proportion (P) at 95% confidence level.									
d	**0.05**	**0.10**	**0.15**	**0.2**	**0.25**	**0.30**	**0.35**	**0.40**	**0.45**	**0.50**
0.05	73	138	196	246	288	323	350	369	380	384
0.10	18	35	49	61	72	81	87	92	95	96

_____d=

Degree of freedom, P=Anticipated population proportion.

Source :(WHO, 2003). Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated *P. falciparum* malaria Geneva.

3.4 Study Design

The study was a one arm prospective evaluation of clinical and parasitological responses to treatment for uncomplicated malaria using artemether/lumefantrine. Children who met the inclusion criteria were enrolled into the study. They were treated with artemether/lumefantrine at the hospital and monitored for 28 days. The patients were followed-up during this period, and their blood samples and clinical conditions were examined. On the basis of the results of their monitoring, they were classified as having early therapeutic failure, late parasitological failure, late clinical failure or adequate clinical and parasitological response. The proportion of patients that showed treatment failure during the follow-up period was used to estimate the efficacy of the drug.

3.5 Duration of the Study

The study was a prospective evaluation of clinical and parasitological responses of uncomplicated *P. falciparum* malaria on days 0, 1, 2, 3, 7.14, 21 and 28. The day a patient was enrolled and received the first dose of medicine was designated as day 0. A follow-up of 28 days was conducted in which the patients were given the study drug for the first three days and monitored clinically and parasitologically throughout the follow-up days.

3.6 Screening Evaluation

Patients were screened at the Paediatrics outpatient unit to identify those who met the enrolment criteria. The most important data captured during screening were address, age, sex, temperature, body weight, and height of patient. Blood smear examination was performed on patients that met the enrolment criteria. The data collected for all screened patients were entered in the case screening form provided (Appendix II). Patients that do not meet the enrolment criteria were not excluded but were treated by facility staff in accordance with the routine practice of the hospital.

Patients that met the basic enrolment criteria further underwent clinical evaluation by the clinician or clinical staff. Special care was taken in evaluating patients with early signs of febrile diseases other than malaria.

3.7 Inclusion Criteria

The following criteria were used for the study:

- Patients between 6 months to 59 months of age.
- Patients must be infected only by *P. falciparum*
- Patients with parasitologically confirmed *Plasmodium falciparum* malaria (asexual parasite 2000-200,000/µl).
- Presence of oral temperature $\geq 37.5\ ^{O}C$ or history of fever during the past 24 hrs.
- Ability to swallow oral medication.
- Ability and willingness to comply with the study protocol for the duration of study and to comply with the study visit schedule
- Voluntary Informed consent from the parent or guardian of children.
- Body weight of ≥ 5 kg
- Absence of severe danger signs or severe *Plasmodium falciparum* malaria according to WHO definition (WHO, 2000). General danger signs of severe or uncomplicated illness are; prostration, impaired consciousness, respiratory distress, multiple convulsions, circulatory collapse, pulmonary oedema, abnormal bleeding, jaundice and haemoglobinuria.
- Absence of severe malnutrition according to WHO child growth standards (WHO, 2006a).

- Absence of febrile conditions due to diseases other than malaria (e.g. measles, acute respiratory tract infection, severe diarrhea with dehydration) or other known underlying chronic or severe diseases e.g. cardiac or hepatic disease.
- Absence of medication that may interfere with the pharmacokinetics of artemether or lumefantrine.
- Absence of history of hypersensitivity to artemether or lumefantrine.

3.8 Exclusion Criteria

- The presence of febrile condition other than malaria or other known underlying chronic or severe disease.
- The presence of danger signs or signs of severe malaria due to *Plasmodium falciparum* according to WHO definition (WHO, 2000).
- The presence of mixed or mono-infection with another Plasmodium specie seen under the microscope.
- Presence of severe malnutrition (WHO.2006a).
- Patient taking other medications which may interfere with the pharmacokinetics of artemether or lumefantrine.
- Patient known to have hypersensitivity or contraindication to artemether or lumefantrine**.**

3.9 Informed Consent

Formal informed consent was obtained from all patients that met the enrolment criteria. Patients were only included in the study when their parents or guardians gave their informed consent. Details about the study and its benefits and potential risks were explained to the patient's parent

or guardian in the native Hausa language. They are requested to sign the consent form (Appendix III) after agreeing to participate in the study.

3.10. Treatment

3.10.1 Antimalarial treatment

Patients were given artemether/lumefantrine 20 mg/120 mg according to body weight or age as recommended by the National Policy on Treatment of Malaria (FMOH, 2005). The artemether/lumefantrine (20mg/120mg) under test was obtained from the Free Antimalarial Drug Programme of Kano State. In total, six doses were administered at 0, 8, 24, 36, 48, and 60 hrs. The dose was administered by Pharmacist and a trained research assistant in the clinic.

The patients were observed for 30 minutes after administration of the drug for vomiting. Patients who vomit after administration of the drug were given another dose and then observed for another additional 30 minutes. Patients that vomits again, were withdrawn from the study and treated with rescue therapy of artesunate or quinine.

3.10.2 Concomitant treatment

In addition to the artemether/lumefantrine given to the patients to treat malaria, paracetamol syrup was also given to the patients to reduce the body temperature. The use of herbal medicines was avoided. However, if any medicine was to be used during the study, the name, date, and time of administration must be recorded in the case report form (Appendix IV).

3.10.3 Follow-up schedule

Patients that were assessed clinically and met enrolment criteria were given an identification number. The research was fully explained to the parents or guardians of the patients before seeking their consent for inclusion into the research.

The follow-up schedule (Appendix V), the case report forms (Appendix IV), and serious adverse events forms (Appendix VI) were used to record the information and clinical observations on each patient enrolled into the research.

The day the patient was enrolled and received the first dose of the treatment was designated as day 0. The treatment was the repeated on days 1and 2. Clinical reassessment was made on days 1, 2, 3 and 7, then on days 14, 21, and 28. Patients were advised to return on any day other than the scheduled days when symptoms of their ailment get worse. Blood film for parasite count was made on days 2, 3, 7, 14, 21, and 28 or on any other day if the patient visits the facility. Blood films were also obtained when parasitological reassessment was required by the clinician for reason of safety of patient.

The initial follow-up days i.e. days 1, 2 and 3 are very critical to both the patient to ensure safety and to the researcher for assessing efficacy of the drug.

Patients were encouraged to return to the hospital for follow up visits. The ultimate success of the research rested on minimizing loss to follow-up. Provision was made ahead of time to locate patients at home if they do not attend as scheduled. Patients who failed to appear on day1 and day 2 and miss one dose of the treatment were withdrawn from the study. After day 3, any patient that failed to appear on day 7 but appeared on day 6 or 8 but absented on day 14, and appeared on day 15, absented on day 21, but present on day 22, absented on day 28 but appeared

on day 29, were still included in the study . Violation of the protocol for more than one day was not allowed, for the safety of the patient and for the relevance of the study data obtained.

3.10.4 Rescue treatment

Intravenous/ intramuscular quinine or artesunate or artemether were made available as rescue treatment. Quinine injection will be given at the dose of 20 mg/kg (as quinine hydrochloride) as a loading dose diluted in 10 ml/kg of 4.3% dextrose in 0.18% saline or 5% dextrose over a period of 4 hrs. This will be followed by 10 mg/kg infusion after 12 hrs. and given over a period of 4 hrs. every 8 hrs. until the patient can take it orally. When the patient can take orally, quinine tablet 10 mg/kg 8 hourly will be given for seven days or artemether -lumefantrine tablets 12 hourly for three days.

Artesunate can be given as 2.4 mg/kg IV bolus, and then 1.2 mg/kg repeated after 12 hrs and subsequently 1.2 mg/kg daily for six days. Artemether can also be given alternatively at a dose of 3.2 mg/kg IM as a loading dose followed by 1.6 mg/kg IM daily for six days. Any patient with signs of severe complicated malaria would hospitalized and given parenteral therapy as stated above with relevant supportive therapy. Any patient that meets one of the criteria for therapeutic failure will received oral quinine 10 mg/kg 8hourly for seven days according to the current National Policy on the Treatment of Severe Malaria.

3.10.5 Loss to follow-up

Those patients lost after they were enrolled into the study were classified as lost to follow –up. Loss to follow- up patients were not included in the analysis. Any patient lost to follow- up but later reappears at the facility before day 28 was not turned away but encouraged to return for check-up visits.

3.10.6 Patient discontinuation or protocol violation.

Patients that met any of the following criteria were withdrawn from the study:

- A patient that withdraws consent at any time without prejudice for further follow- up or treatment at the facility.
- A patient who vomits the medication twice will be withdrawn.
- Failure to attend scheduled visits during the first three days.
- Serious adverse reactions prompting the termination of treatment before day 28.
- Patient with severe malaria at day 0 erroneously enrolled.
- Self-medication or third party administration of antimalarial drug or antibiotic with antimalarial activity.
- Detection of another malaria specie during follow –up.
- The occurrence of another disease that could interfere with the classification of treatment outcome.

3.11 Study End Points

The classification assigned to a patient marks the end point of the study. Study end points includes treatment failure, completion of follow-up period without treatment failure, loss to follow-up, withdrawal from study, and protocol violation.

3.11.1 Efficacy evaluation

Treatment outcome was classified on the basis of an assessment of the parasitological and clinical outcome of the antimalarial treatment according to the WHO guideline (WHO, 2009). All patients were classified as having either early treatment failure; late clinical failure, late

parasitological failure or adequate clinical and parasitological response. Patients who showed treatment failure were given rescue treatment as described earlier on. Follow up was continued until the patient fully recovered.

3.11.2 Safety evaluation

All patients were asked about previous symptoms and about symptoms that have emerged since the previous follow up visit. When clinically indicated, patients will be evaluated and treated appropriately. Adverse drug reaction were recorded on the case report form (Appendix IV)

3.11.3 Quality evaluation

The results of the disintegration time, weight uniformity, crushing Strength and friability tests were used to determine the quality of the tablets.

3.11.3.1. Disintegration time:

Basket rack assembly method (B.P. 2009)

In conducting the disintegration test, one tablet was placed in each of the six tubes of the basket-rack. Distilled water was used as the immersion fluid and maintained at a temperature of 37 ± 2 ^{O}C. The apparatus was operated and the basket-rack assembly containing the tablets was raised and lowered at a constant frequency in to the immersion fluid by an automated device. The apparatus was left to run for 15 mins. At the end of the 15 mins the basket-rack assembly was

raised from the fluid and the tablets were observed for disintegration. To pass the test all six tablets must disintegrate completely within 15 mins If one or two tablets failed to disintegrate completely, the test will be repeated on 12 additional tablets. The requirement will be met if no fewer than 16 of the total of 18 tablets tested disintegrates.

3.11.3.2. Weight uniformity test (B.P.2009).

In testing for uniformity of weight, 20 tablets were weighed individually using analytical balance. The average weight of the twenty tablets was determined from their individual weights. The deviation of the individual weight of each tablet from the average weight should not exceed the limit of ± 7.5 with a minimum number of 18 tablets, each weighing 80-250mg.

3.11.3.3. Crushing strength test (B.P. 2009)

10 tablets were subjected to crushing effects of the two edges of the Mosanto machine. The tablets were compressed between the two platens of the tensile tester and the force expanded to crush the tablet was recorded.

3.11.3.4. Friability test (B.P.2009)

10 tablets were used to perform the friability test. They were initially weighed and the weight recorded. They were subjected to abrasion using a Vego tablet friability tester at 25 Rev/min and then the final weight noted again.

3.11.4 Clinical evaluation: All patients were evaluated clinically as described below.

3.11.4.1. Physical examination:

Physical examination was done at baseline by a trained hospital personnel i.e. day 0 before dosing and days 1, 2, 3, 7, 14, 21, and 28. A complete medical history, presence of danger signs, axillary temperature, demographic information and contact detail were taken at baseline.

3.11.4.2. Body weight:

Body weight was measured using a weighing balance and the weight recorded on day 0. Excessive clothing were removed from the patients to avoid over estimating their true weight. The measured weight was used to satisfy the inclusion or exclusion criteria for nutrition status and to calculate the dose to be administered.

3.11.4.3. Body temperature:

Body temperature was measured at baseline i.e. day 0 before dosing and on days 1, 2, 3, 7, 14, 21, and 28 using a clinical thermometer. Temperature was measured with a thermometer that has a precision of 0.1 $^{\circ}$C.

3.11.4.4. Preparation of blood films for malaria parasite count:

Slides to be used were cleaned with methanol and soaked in water containing detergent overnight. They were then washed with clean water and left to dry.

Thin films were prepared by collecting blood from any of the fingers by pricking the finger with a lancet. The blood was placed at the middle of the slide. A second slide was held at an angle of 45^{O}C to the slide containing the blood and the blood was pulled along the length of the slide to give a thin film. A thick film was prepared by making another drop at the end of the same slide or different slide. Using the end of another slide an oblong shape film was made.

3.11.4.5. Microscopic blood examination and counting

Thick and thin blood films for parasite counts were obtained and examined at screening on day 0 to confirm adherence to the inclusion and exclusion criteria. Blood films were also examined on days 2, 3, 7, 14, 21, and 28 or any other day if the patient returns spontaneously and parasitological reassessment is required. Specimens slides were labeled with the study number, day of follow-up and date.

Giemsa stain of dilution 2.5% and 10% were prepared and two blood slides per patient were obtained. One slide was stained rapidly with 10% giemsa for 10-15 minutes for initial screening, while the other was retained. When the patient was finally enrolled, the second slide was stained more carefully with 2.5% giemsa for 45-60 minutes and slower staining was used for all the slides obtained at follow-up visits. Giemsa stained thick and thin blood films were examined at a magnification of x1000 to identify the parasite species and to determine the parasite density.

. The study number of the patient, the date, and the day of follow-up were recorded on the edge of the slide with a permanent marker. Thick blood smear for initial screening was used to count the number of asexual parasites and white blood cells in a limited number of microscope fields. The adequate parasitaemia for enrolment was at least one parasite for every three white blood cells, corresponding to approximately 2000 asexual parasite per micro litres of blood. The second blood smear was used to calculate the parasite density, by counting the number of asexual parasites in a set of number of white blood cells typically 200 with a hand counter. Once a field has been started, it must be counted to completion; the final number of white blood cells were rarely exactly 200. If more than 500 parasites were counted before 200 white blood cells have been reached, the count was stopped after the reading of the last field has been completed. Parasite density expressed as the number of asexual parasite per µl of blood, was calculated by dividing the number of asexual parasites by

the number of white blood cells counted and then multiplying by an assumed white blood cells density typically 8000 per µl.

$$\text{Parasite density (per/µl} = \frac{\text{Number of parasite counted} \times 800}{\text{Number of leucocytes counted}}$$

The same technique was used to establish the parasite count on each subsequent blood films. When the number of asexual parasite was less than 10 per 200 white blood cells in the follow-up smears, counting was done against at least 500 leucocytes (i.e.to completion of the field in which the 500^{th} white blood cell is counted). A blood slide was considered negative when examination of 1000 white cells reveals no asexual parasites. The presence of gametocytes on an enrolment or follow-up slide was noted, but this information did not contribute to basic evaluation. Also, 100 fields of the second thick film was examined to exclude mix infections. In the case of any doubt the thin film was examined for confirmation. If examination of the thin film was not conclusive, the patient was excluded from the analysis after complete treatment and follow-up.

A qualified microscopist read all the slides independently and parasite densities were calculated by averaging the two counts. Blood smear with discordant results were re-examined again by the microscopist and parasite density was calculated by taking the average of the two closest counts.

3.12 Ethical Clearance

Ethical clearance was obtained from the Kano State Hospitals Management Board before conducting the study (Appendix I).

3.13 Data Analysis

Data was analyzed using graph pad instat for windows version 15. Descriptive statistics as percentages, mean, median, standard deviation and range were applied. The efficacy assessment was done by the modified intention to treat and per protocol analysis. The intention to treat population was used for analysis of variables, primary and secondary efficacy end points. The *per protocol* employed the Kaplan-Meier for analysis of primary outcomes (Early treatment Failure, Late clinical failure, Late parasitological failure and Adequate clinical and parasitological response). Bivariate analysis was used to compare means.

3.14 Limitation of the study

- The study drug was not administered with milk or fatty foods which are believed to improve the bioavailability of the drug.
- Therapeutics blood monitoring was not done
- Quantitative analysis could not be performed on the tablets

CHAPTER FOUR

4.0 RESULTS

A total number of 145 patients screened for uncomplicated *P. falciparum* malaria, out of which 73 (50.3%) were found to be positive for P. *falciparum* and fulfilled the inclusion criteria by WHO. The number of patients that completed the 28 days follow-up was 63. A total of 10 patients were lost to follow-up on different days such as five on day 1, two on day 2, two on day 3, and one on day 14, but no withdrawals was recorded. Figure 4.1 shows summary of the study of treatment with artemether/lumefantrine of children under 5 with P falciparum malaria.

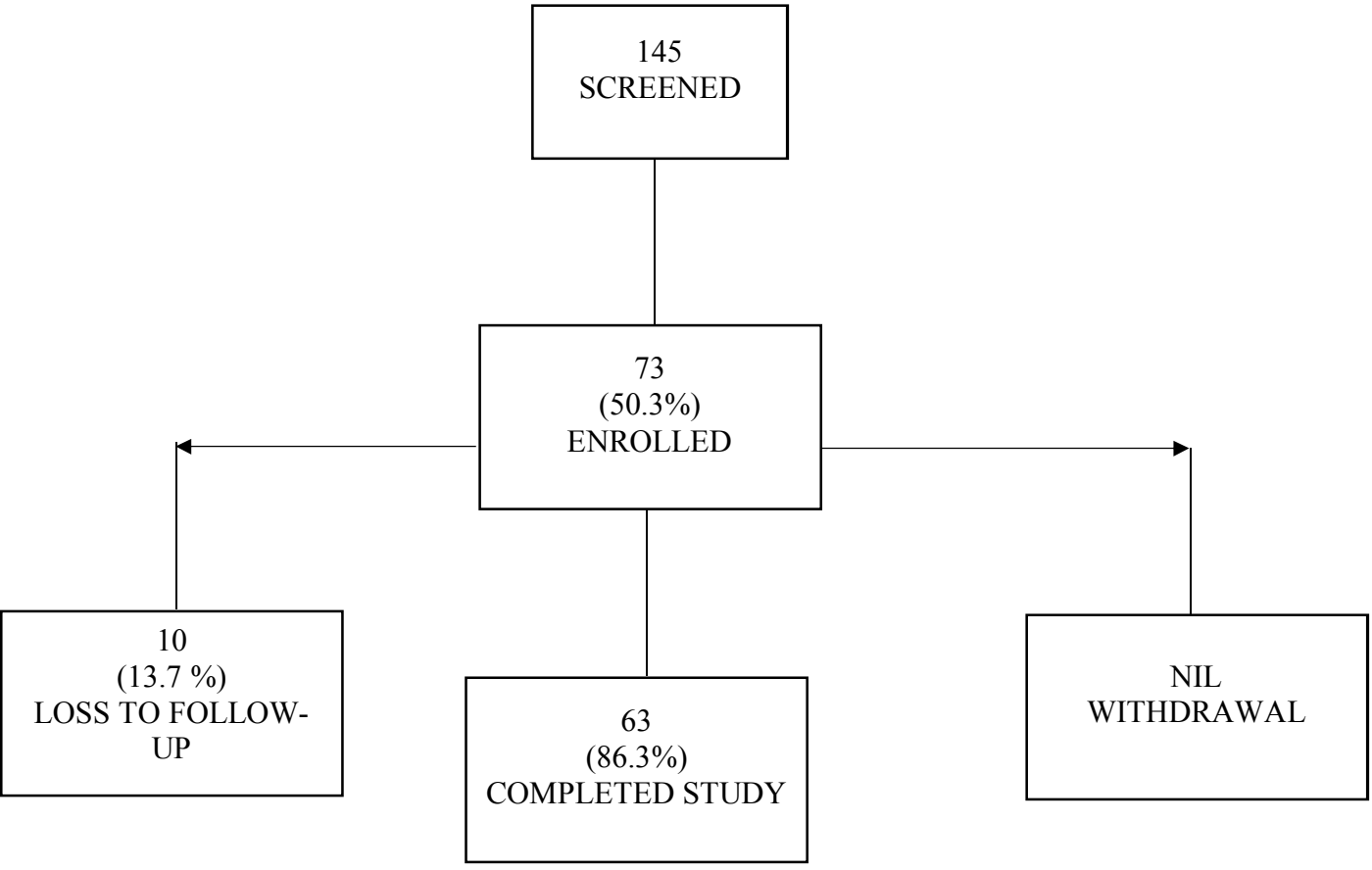

Figure 4.1 Summary of the study on the treatment of uncomplicated P. *falciparum* Malaria in children under 5 years with artemether/lumefantrine

4.1 Baseline Characteristics of Study Population at Enrolment

From the total number of patients that were screened, 57.6% were male, while 42.4% were female. The mean age, weight and height of the study population were found to be 29.75 months, 10.31kg, and 78.14 cm respectively. The class range for age, weight and height was 6-59 months, 5-22 kg and10-108 cm respectively. The mean body temperature of the study group was 37.6 $^{\circ}$C with a class range of 34.5 $^{\circ}$C to 40 $^{\circ}$C. The mean parasite density was 3678 cells/µl with also a range of 2001-14000 cells/µl. Table 4.1 below shows the baseline characteristics of the study population.

Table 4.1: Baseline Characteristics of the Study Population

Variables	Mean ± SEM	Range
Age (months)	29.75 ± 1.95	6-59
Bodyweight (kg)	10.31 ± 0.45	5-22
Height (cm)	78.14 ± 1.84	10-108
Temperature ($^{\circ}$C)	37.60 ± 0.11	34.5-40
Parasite density (µl)	3768.00 ± 265	2001-14000

4.2 Fever and Parasite Clearance Times

The mean fever clearance time was 40.22 hrs. The fever completely disappeared in 45.63 hrs. time in all the patients. The parasite clearance time was 52.14 hrs. and the parasites were completely cleared also in all the patients in 66.58 hrs. This means that by day 3, all the patients had no fever or parasites. Table 4.2 shoes the parasite count, fever and parasite clearance times at enrolment.

Table 4.2 Parasite Count at day 0, Fever and Parasite Clearance Times

Variables	Mean ± SEM	Range
Parasite count at day 0 (µl)	3768 ± 265	2001-1400
Fever clearance time (hr.)	40.22 ±2.86	33.53-45.63
Parasite clearance time (hr.)	52.14 ± 5.07	43.28-66.58

4.3 Treatment Response

The Kaplan Meier survival analysis of the data showed estimates of success cumulative incidence of 1.00 from day 0 to day 6, 0.941 from day 7 to day13, 0.911 from day 14 to day 20, 0.896 from day 21 to day 27, and 0.864 on day 28. (Figure 4.2).

The failure cumulative incidence was 0.00 from day 0 to day 6, 0.059 from day 7 to day 13, 0.089 from day 14 to day 20, 0.104 from day 21 to day 27 and 0.136 on day 28.

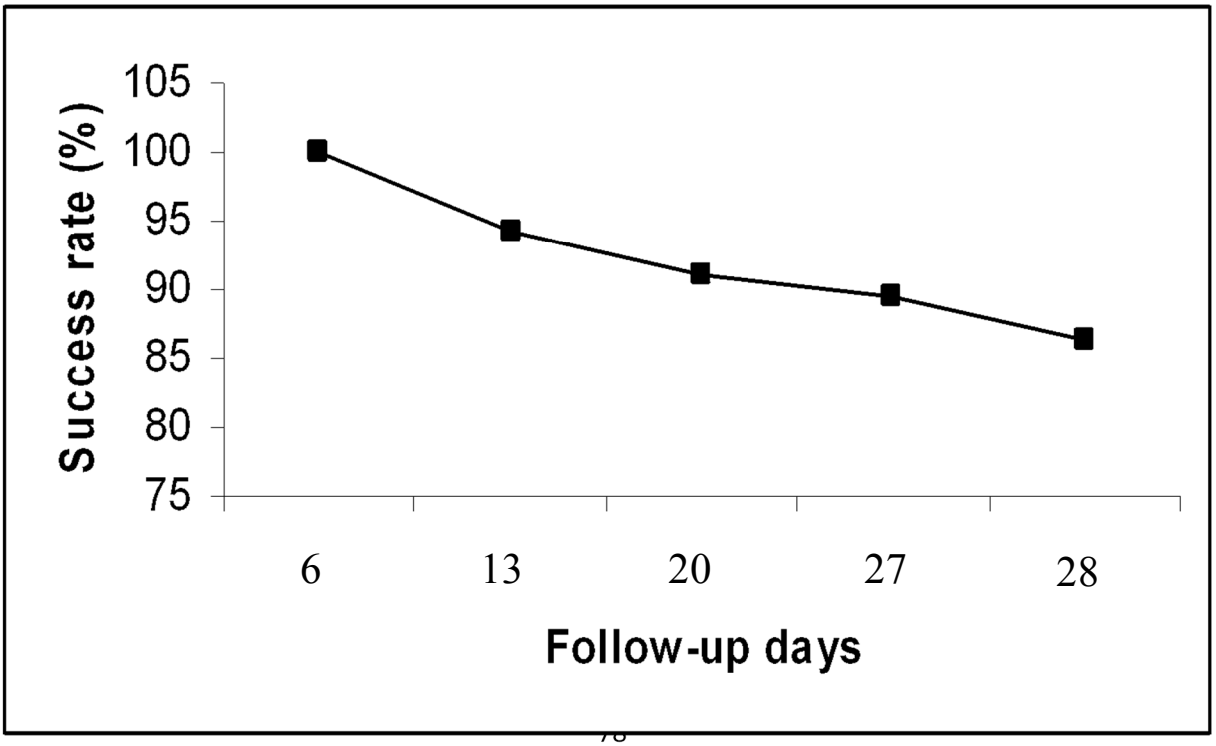

Figure 4.2 Success rate in the treatment of children under 5 years of age with P. *falciparum* Malaria with artemether/lumefantrine by follow-up days

4.4 Classification of Treatment Outcome According to WHO, 2009

The study outcome by per protocol analysis showed that there was no early treatment failure. However, there was one late clinical failure, eight late parasitological failures and 54 adequate clinical and parasitological responses (Table 4.3) The efficacy observed was 85.7% PCR uncorrected (Table 4.3 and Figure 4.3) at 0.746 to 0.933 confidence level and failure rate of 14.3%. The study outcome by follow-up days showed 0% at day 7, 1.6% at day 14, 12.7% at day 21 and 85.7% at day 28. Table 4.3 below shows treatment outcomes based on WHO 2009.

Table 4.3 Treatment Outcomes based on WHO 2009

Clinical Outcome	Number of Patients	Percentage (%)
ETF	0	0
LCF	1	1.6
LPF	8	12.7
ACPR	54	85.7
Total Analysis	63	100

ETF = Early Treatment Failure

LCF = Late Clinical Failure

LPF = Late Parasitological Failure

ACPR = Adequate Clinical and Parasitological Response.

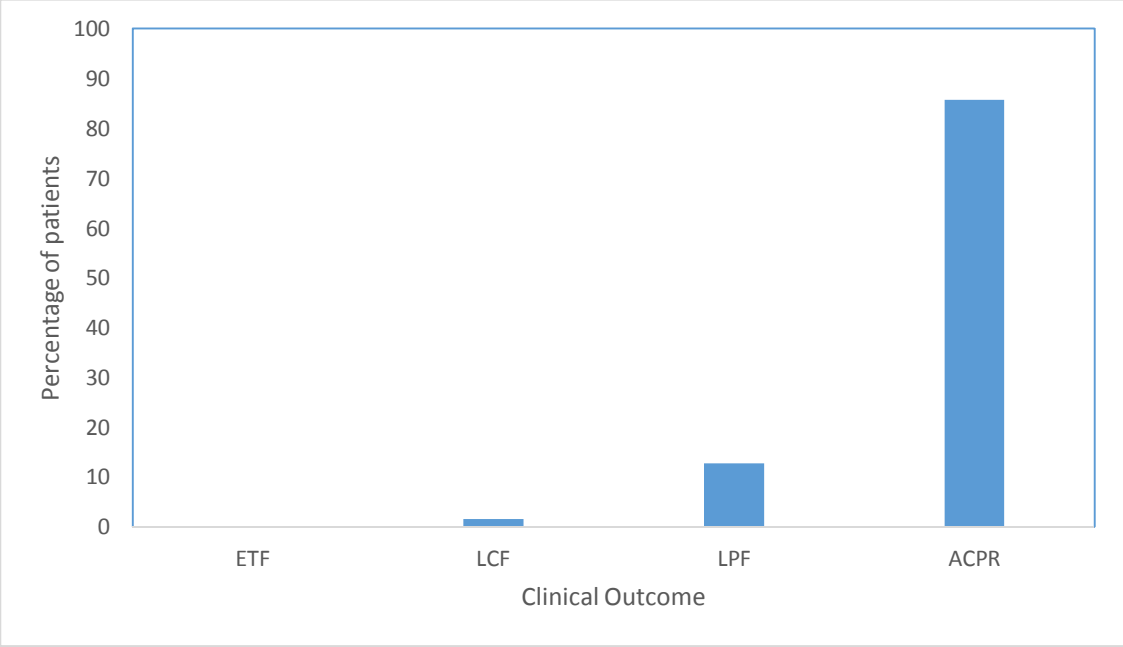

Figure 4.3 Classification of treatment outcome in the treatment of *P falciparum* malaria In children under 5 years of age with artemether/lumefantrine

Key:

ETF = Early Treatment Failure, LCF = Late Clinical Failure

LPF = Late Parasitological Failure, ACPR = Adequate Clinical and Parasitological Response

4.5 Qualitative Tests on Artemether/Lumefantrine

The results of the qualitative tests showed that the disintegration time for the tablets was 4.5 min, while the weight uniformity, crushing strength and friability were 244.5mg±1.1, 9.05Kg F and 0.4% respectively as shown in table 4.4 below.

Table 4.4 Result of Qualitative Tests on Artemether/Lumefantrine tablets

Variables	Values
Disintegration time	4.5 minutes
Weight uniformity	244.5mg ± 1.1
Crushing strength	9.05 Kg F
Friability	0.4%

CHAPTER FIVE

5.0 DISCUSSION

The treatment of choice for uncomplicated *P. falciparum* malaria is Artemisinin Based Combination Therapy (ACT) (WHO, 2006). ACTs are combinations consisting of an artemisinin derivative and another effective long acting schizonticidal antimalarial drug. The recommended ACTs for the treatment of uncomplicated *P. falciparum* malaria in Nigeria are Artemether/Lumefantrine and Artesunate/Amodiaquine (FMOH, 2005). Artemether/Lumefantrine combination contains 20 mg artemether and 120 mg lumefantrine. The combination is given according to body weight and age of the patient at 0, 8, 24, 36, 48, and 60 hrs. Artesunate/Amodiaquine combination contains 100 mg artesunate and 270 mg of amodiaquine. It is also given according to body weight and age. It is given once daily for three days.

This study was conducted to assess the therapeutic efficacy, safety and quality of artemether/lumefantrine supplied by the Kano State Government for the treatment of uncomplicated *P. falciparum* malaria in children less than 5 years of age. The sample size of the study population was 73 patients. This consist of 57.6% male and 42.4% female. The proportion of male and female patients was found to be 51.9% and 48.1% respectively in a study conducted on African infants and children by Falade *et al*, (2005). In another study conducted among children less than 5 years by Salah *et al* (2006), the proportion of male patients was 68.2% while the female was 31.8%. The higher proportion of male patients than the female patients in our study and the above mentioned studies is not related to disease prevalence but rather male patients turn out at the facilities.

The mean temperature of the study population at recruitment was 37.6 °C ± 0.11. In another study conducted among children less than 5 years, the mean temperature of the study group was 38^{o}C ± 0.3 (Assefa *et al,* 2010). In a similar study conducted also by Gebremedhin *et al* (2012), the mean temperature recorded was 38.2^{o}C ± 0.86. The relatively lower temperature observed in our study population could be as a result of the fact that the study was conducted in the month of November during the harmattern season.

The mean parasite density of the study group was 3768 cells/µl ± 265 with a range of 2001 and 14000 cells/µl.

The study showed a mean fever clearance time of 40.22 hrs and mean parasite clearance time of 52.14 hrs. The fever clearance time is the time between commencing treatment and the temperature returning back to normal and remaining normal for more than 48hrs, while the parasite clearance time is the t me between commencing treatment and the first negative blood test, when negativity persist for more than 48hrs.The ranges for fever clearance time and parasite clearance time were 33.53 - 45.65 hrs and 43.28 - 66.58 hrs respectively. In another study, the mean fever clearance time and parasite clearance time were 26 hrs and 26.5 hrs respectively (Gebremedhin *et al,* 2012). In another study conducted to assess the efficacy of artemether/lumefantrine for uncomplicated malaria the fever clearance time was 47 hrs and parasite clearance time of 36 hrs respectively (Omari *et al,* 2004). The differences in fever clearance time in different areas may be due to differences in parasite density at enrolment. The fever clearance time was shorter than the parasite clearance time in our study and that reported by Gebremedhin *et al,* (2012). This is thought to be due to rapid absorption and onset of action and the antipyretic properties of artemether (Lefevre, 1999). It is also found to clear the fever rapidly through it action on the blood stages of the malaria parasite (Premji *et al*, 2009).The

lumefantrine concentration persist in the blood after day 2, eliminating any residual parasites to prevent recrudescence (Bloland, 2000). However, the relatively higher fever clearance time in our study compared to the above mentioned studies could be due to lack of intake of fatty food along with the drug as fatty food or milk improves bioavailability of artemether by more than two folds and lumefantrine by more than sixteen folds (Ashley *et al*, 2007). There were 72% of the patients that had there fever cleared completely in day 1 while 88% and 91% of the patients in days 2 and 3 respectively. The fever and the parasites were cleared from all the patients within 72 hrs of commencement of treatment in our study. This is consistent with the study carried out by Assefa *et al,* (2010).

The treatment outcome from the analysis showed that there was no early treatment failure, but there was one clinical failure, eight parasitological failure, and fifty four adequate clinical and parasitological response were record. In a similar study conducted in Kaduna State North-West of Nigeria, there was no early treatment failure, but one late clinical failure, one late parasitological failure and fourty one adequate clinical and parasitological response were recorded out of fourty three patients (Umar, 2014). The absence of early treatment failure indicates that no patient developed danger signs or severe malaria on days 1, 2 or 3 in the presence of parasitaemia and there was no parasitaemia on day 2 or day 3 irrespective of axillary temperature or with temperature greater than 37.5^{o}C (WHO, 2009). The presence of one late clinical failure indicates that there was parasitaemia on any day between day 4 and day 28 with axillary temperature greater than 37.5^{o}C or history of fever in patients who did not previously met any of the criteria for early treatment failure. (WHO, 2009). Also the presence of eight late parasitological failure signifies that there was eight pants with parasitaemia between day 7 and 28. The absence of early treatment failure and the one late clinical failure of the two studies are

similar. The difference lies at the late parasitological failures. The study conduct by Umar (2014) had 2.3% of the total analysed cases as late parasitological failure, while our study had 12.7% of the total analysis as late parasitological failure. The higher late parasitological failure is responsible for the relatively lower efficacy obtained compared to what was reported by umar (2014).

The study result also showed an efficacy of 85.7 % (PCR uncorrected) in 28 days as per the *per protocol* analysis and a failure rate of 14.3%. This result of adequate clinical and parasitological Response (ACPR) is however similar to previous studies (88.7%) conducted jointly in the African infants and children (Falade *et al*, 2005). The cure rate for the intention to treat analysis (ITT) was 86.3% for the brand of artemether /lumefantrine investigated in 28 days. This figure is also similar with the studies conducted on African infants and children (86.5%) for the intention to treat analysis (Falade, *et al*, 2005).

The cure rate of the drug on day 14 was 91.1 %. This is consistent with *in-vivo* response of the *P. falciparum* to artemisinin derivatives which were shown to have more than 90% cure rate on day 14 when they were first introduced as first line drug for treatment in Nigeria (FMOH, 2005).

The result of the Kaplan Meier analysis showed that there was cure rate of 100% from day 0 to day 6. This is evident by the absence of early treatment failure. The cure rate begins to decrease gradually to 94.1% then 91.1% and 89.6% from the second, third and fourth week respectively reaching 86.4% by the 28^{th} day of the study. A similar study carried out showed 100% cure rate in both first and second week and 88.7% cure in the fourth week of the study (Falade *et al,* 2005). This shows that as the therapeutics concentration of the drug decreases with time, the cure rate also decreases.

Artemether/lumefantrine had 100% efficacy in the North-West of Nigeria when initially approved as first line treatment for uncomplicated *P. falciparum* malaria as a result of therapeutic efficacy studies conduct in 2002 and 2004 in the six geopolitical zones of the country. In a similar study conducted in Children less than 5 years of age in Kaduna State of the North- West of Nigeria, 95.3% efficacy was observed (Umar, 2014). When compared with our figure of 85.7% obtained, it can be seen that the efficacy of artemether /lumefantrine is gradually diminishing from 2005 when initially approved and deployed for use to the present study in 2014. Resistant to antimalarials has been documented for *P. falciparum, P. malariae, and P. vivax.* Resistance in *P. falciparum* has been observed in currently used antimalarials such as amodiaquine, mefloquine, quinine, and Sulfadoxine/Pyrimethamine and more recently artemisinin derivatives (WHO, 2010). The emergence of resistance is considered in two ways; the initial genetic event that produces the resistant mutant and secondly the subsequent selection process in which the survival advantage in the presence of the antimalarial drug leads to preferential transmission and the spread of resistance (White, 2002). Wide spread and indiscriminate use of antimalarial drugs places a selective pressure on malaria parasite to evolve mechanisms of resistance (WHO, 2010). Predicting the emergence and spread of resistance to current antimalarials in use is necessary for planning malaria control and instituting strategies that might delay the emergence of resistance.

There was no serious adverse event observed during the 28 days follow-up. The recorded side effects were vomiting and diarrhoea. This could also be symptoms of malaria (WHO, 2000) or could as well be side effects of the artemether/lumefantrine co-formulation (WHO, 2010).

The results of the qualitative tests performed on the test tablets were all within normal or official limits of the various tests. The disintegration time for the test tablets was 4.5 min.

The B.P.2009 official value of disintegration time for uncoated tablets is that all the tablets should disintegrate within 15 minutes when subjected to disintegration test and for film coated tablets, all the tablets should disintegrate within 30 minutes. The disintegration time of 4.5 minutes can be said to be well below the official value of 15 min. In a similar study conducted on substandard artemisinin-based antimalarial medicines in licensed retail pharmaceutical outlets in Ghana, a disintegration time of 3.83 minutes was obtained (El-Duah *et al*, 2012). From the disintegration test, it is possible to understand the *in- vivo* dissolution variance as a function of tablet's physical properties. The disintegration time measures the ability of particles of the tablets to de aggregate in to granules and subsequently dissolve into solution. The dissolved particles releases the active medicaments making it bioavailable for absorption and subsequent therapeutic action. Although disintegration is not the rate determining step of dissolution, the faster the tablet disintegrates into granules, the faster the bioavailability of the active drug.

The average weight of the 20 tablets was determined to be 244.5 mg ±1.1. The B.P. 2009 official limit for weight uniformity of tablets is that the deviation of the individual tablet from the average weight should not exceed the limit of ± 7.5 with a minimum number of 18 tablets and each tablet weighing between 80 mg to 250 mg.

The weight uniformity test conducted in the comparative analysis of the physicochemical properties of five brands of artemether/lumefantrine tablets was 240 ±1.6 mg (Mukesh, 2011). In another similar study conduct, the average weight obtained was 244.1±1.7mg (El-Duah *et al*, 2012).

The deviation of the weight of the lowest tablet from the average was ± 4.5 and that of the highest weight from the average was ± 5.5. The result of our study is consistent with B.P. 2009

official specification and other similar studies carried out in other areas. This shows that the weight uniformity of the tablets is in compliance with qualitative standards required of good tablets. The concept of preparing of powders into granules during manufacturing and then granules compressed into tablets emphases the importance of weight uniformity. Tablets with uniform weights are expected to have uniform active medicaments. This avoids under dosage or over dosage during treatment with the same batch of tablets. The crushing strength of the test tablets was 9.05 Kg F. This was also within the official limit of 4-14 Kg F of B.P.2009 for crushing strength of tablets. In a study of substandard artemisinin-based antimalarial medicines, the crushing strength of the samples of artemether /lumefantrine obtained was 11.2Kg F (El-Duah *et al*, 2012). The tablets can be said to have complied with standards for good quality tablets. The crushing strength is an essential criteria in the determination of the ability of the tablets to resist chipping, abrasion or breakage under conditions of storage, transportation and handling before storage. It measures the tensile strength of the tablets to resist external forces or humidity.

The friability tests obtained for the test tablets was 0.4% .The B.P. 2009 limit for friability test for qualitative tablets is less than 1%. In a study of the comparative analysis of physicochemical properties of five brands of artemether/lumefantrine, the friability observed was 0.01% (Mukesh, 2011). Although this result is much smaller than our study result of 0.4%, the friability is still with the limit of the official value of the B. P 2009. Friability test is also a measure of the level of compliance to standard qualitative manufacturing practice. It is the measure of the ability to withstand handling properties of tablets. Poor compliance to friability standards leads to breakage of tablets during transportation. This may leads to loss of active drug. The tablets used in this study have passed the friability test. Thus the tablets can be said to be of good quality.

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

From the total number of 145 patients with fever that were screened for uncomplicated *Plasmodium falciparum* malaria, 50.3% that fulfilled the inclusion criteria were enrolled in to the study. The enrolled study population consisted of 57.6% male and 42.4% female. The number of patients that completed the study up till day 28 were 63 (86.3%), while 13.7% were lost to follow-up. There was no withdrawal observed from the study and no patient developed severe malaria.

The mean temperature of the study population was 37.6 °C ± 0.11 with temperature range of 34.5-40 °C. The mean parasite count of the study population was 3678 ± 265 cellsμ/l with parasite density range of 2001 and14000 cellsμ/l of cells. The mean fever clearance time was 40.22 hrs.and the range for fever clearance was 33.53-43.28 hrs. The mean parasite clearance time was 52.14 hrs.

35 out of the patients (48%) showed temperature at recruitment equals or less than 37 °C while 4 patients (5.5%) had temperature greater than 39.1 °C. Also 50 patients (68.5%) had parasites count at day 0 between 2000-4000μ/l of cells.

The Kaplan Meier survival analysis showed estimates of success cumulative incidence of 1.0 from day 0 to day 6, 0.941 from day 7 to day 13, 0.911 from day 14 to day 20, 0.896 from day 21 to day 27, and 0.864 on day 28.

The study outcome by *per protocol* analysis showed that there was no early treatment failure. However there was 1 Late Clinical Failure, 8 Late Parasitological failure and 54 Adequate Clinical and Parasitological Response. This showed that the efficacy of the test drug was 85.7% PCR-uncorrected.

The results of the qualitative tests were all within the official limit of B.P. 2009.The determination of the disintegration time showed a time of 4.5min, while the average weight of the tablets was 244.5 mg ±1.1. For any tablet to pass this test, the deviation of the individual weight from the average weight should not exceed the limit of ± 7.5 with a minimum number of 18 tablets and each weighing between 80 mg to250 mg. The test tablets showed a crushing strength of 9.0 kg F, while the determined friability of the tablets was 0.4 %.

The study showed that the standard six dose regime in malaria treatment with the brand of artemether/lumefantrine was efficacious, safe and qualitative in the treatment of uncomplicated *P. falciparum* malaria in children less than 5 years of age with an efficacy of 85.7% PCR uncorrected in the 28 days per protocol analysis.

6.1 RECOMMENDATIONS

From the study it is recommended that:

- the therapeutic efficacy, safety and quality of this drugs needs to be routinely monitored to be able to detect treatment failure as a result of development of resistance to the drug.
- comparative studies be carried out on the therapeutic efficacy of the drug with other artemisinin based drugs used in the area.

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APPENDICES

Appendix I

ETHICAL APPROVAL

KANO STATE
HOSPITALS MANAGEMENT BOARD
BOARD HEADQUARTERS
P.M.B 3540, Post Office Road, Kano

HMB/GEN/488/VOL.I **24/11/1434AH, (30/09/2013)**

Muhammad Auwal Muhammad
Faculty of Pharmaceutical Science,
Ahmadu Bello University,
Zaria.

PROVISIONAL ETHICAL CLEARANCE

Sequel to conduct research title **"CLINICAL OUTCOME OF TREATMENT OF UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA WITH ARTEMETHER LUMEFANTIRINE IN CHILDREN UNDER 5 YEARS in M. Muhammad S. Hospital).** In the light of the above, I am mandated to convey provisional clearance to proceed on your study based on the following conditions.

i. That the consent of all participants must be obtained by filling inform consent form.
ii. That you should liase with the Management of the Facility for appropriate guidance.
iii. That any publication related to the study should be brought to the knowledge of the Ethical Committee for approval.
iv. That a copy of your finding should be submitted for documentation, record and final approval, please.

Best regards.

ZAHRA SULEIMAN
Asst. Sec. I. (Estb).
FOR: EXECUTIVE SECRETARY

Cc:

The Chief Medical Directors,
M. Muhammad S. Hospital,
Kano.

Above is for your information and noting, please.

ZAHRA SULEIMAN
Asst. Sec. I. (Estb).
FOR: EXECUTIVE SECRETARY

Appendix II

CASE SCREENING FORM

Case Screening form	
Name of hospital Town: LGA: State	Study number Patient screening number Date of visit (dd-mm-yyyy).
Demographic data	
Date of Birth (dd-mmm-yyyy): or estimated age: in: mon☐ years ☐ Height (cm) Weight (Kg) Sex: ☐ Male ☐ Female	
Pre-treatment temperature	
History of fever in previous 24hr? ☐Yes ☐No Temperature ^{0}C Axillary☐ Tympanic ☐ Rectal☐ Oral☐	
Thick and thin blood smears for estimation of *P.faciparum* parasitic counts	
Species: *P.faciparum*☐ *P.Vivax*☐ *P.ovale* ☐ *P.malariae*☐ Were species other than *P. falciparum* present? if yes, patient is not eligible Approximate no of P *falciparum* asexual parasites; Presence of 1-100 parasites/ 3-6 white cells ☐Yes ☐No (If no, patient is not eligible Presence of P.*falciparum* gametocytes? ☐Yes ☐No	

Hemoglobin: g/dl haematocrit: %

Inclusion criteria

- Age between 6 and 59 months;
- Mono-infection with P.*faciparum* confirmed by positive blood smear (i.e no mixed infection);
- Parasitaemia between 2000 and 200,000 /µl of asexual forms ;
- Measured temperature ≥37.5^{o}C or history of fever within previous 24h;
- Ability to swallow oral medication;
- Ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule ;
- Absence of severe malnutrition according to WHO child growth standard(WHO,2006) (defined as per protocol);

Does the patient meet all the inclusion criteria? ☐ Yes ☐ No (If no, patient is not eligible)

Exclusion Criteria

Signs and symptoms of severe or complicated malalria rewuiring parenterl treatmeral treatment accoridng WHO criteria ;

Mixed or mono-infection with another *Plasmodium* species detected by micrascopy;

Severe malnutrition;

* Febrile conditions caused by diseases other than malaria or other knows underlying or

severe diseases;

* Regular mediciation which interfers with antimalrial phamacokinetics;

* History of hypersensitivity reactions or contraindications to the medicine tested .

Does the patient meet any of the exclusion criteria? ☐Yes ☐No (If yes, the patient is not eligible)	
If yes pleas specify the reason for exclusion:	
Patient informed consent	
Consent form signed: Yes No	Patient identify number: Dated (dd-mmm-yyy):

Source: Adopted from WHO (2003). Assessment and Monitoring of Antimalarial Drug Efficacy for the Treatment of P. falciparum Malaria**.**

Appendix III

INFORMED CONSENT FORM

Name of principal investigator: - Muhammad Auwal Muhammad

Name of organization: - Hospitals Management Board, Kano.

Name of proposal: - Study on the efficacy and safety of artemether /lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria among children under five years of age in Kano, Nigeria.

PART 1

Information sheet.

My name is Pharm Muhammad Auwal, a postgraduate student at Ahmadu Bello University Zaria, who works with the Hospitals Management Board, Kano. I am undertaking a study on the treatment of malaria. Malaria is a dangerous disease; however it can be treated with medicines. The purpose of this study is to confirm the efficacy of the Artemether/lumefantrine issued to you in treating malaria.

We are inviting children below 5years of age living in this area to take part in this study.

I am going to give you information and invite you to consent to have your child participate in this study. Before you decide whether you want your child to participate, you can talk to anyone you feel comfortable with. There may be some words that you do not understand. Please ask me to stop as we go through the information, and I will take time to explain. If you have questions later, you can ask me or the study doctor or the staff.

Your decision to have your child participate in this study is entirely voluntary. If you choose not to consent, all the services your child receives at this clinic will continue as usual. Even if you agree now but decide to change your mind and withdraw later, the services your child receives at the clinic will continue.

Your child will receive 6 doses of medicine over 3days.The medicine artemether/lumefantrine is recommended by the Federal Ministry of Health. As the parasite that cause malaria can become resistant to the medicine, it is important that studies are regularly carried out to make sure the medicine is still working. The medicine is made by. IPCA Laboratories Ltd. This medicine is known to be very effective, but you should know that it has some minor side effects which will be explained to you. If we find that the medicine is not working, we will use what we call rescue medicine to treat you condition.

During follow-up, a small amount of blood will be taken 7 times from your child's finger. Your child may experience a bit of pain or fear when the finger is pricked. The pain should disappear within 1 day. The blood will be dropped onto a slide. The blood will be used to study the malaria in your child's blood. The examination of the blood samples will be done after the study and it will not affect the success of the treatment. Nothing else will be done with the blood.

The study will take place over 28 days. During that time, your child will have to come to the hospital for 1 hr. each day for 7 days. At the end of one month, the study will be finished. At each visit your child will be examined by a physician. You may stay with you child during each of the visit and during the procedures.

On the 1st day, we will take blood for testing. After the tests your child will receive the first dose of the treatment.

On the 2nd visit, your child will receive the second dose of the treatment

On the 3rd visit, your child will receive the third dose of the treatment plus a blood test.

On the4th, 5th, 6th, 7th and 8th visits, your child will have a blood test.

Medicine can have some unwanted or unexpected effects; however, we will follow your child closely and keep track of these effects. We will give you a telephone number to call if you notice anything out of the ordinary, or if you have concerns or questions. You can also bring your child to the hospital at any time and ask to see the doctor. If your child experiences side effects, we may use some other medicine free of charge, which will help to reduce the symptoms or reactions or we may stop one or more of the medicines. If this is necessary we will discuss it together. You will always be consulted before we move to the next step.

If you decide that you child will participate in this study, any illness related to malaria or to the malaria treatment will be treated at no charge to you. Your child's participation will help us to make sure the medicine is still working and this will benefit society and future generations.

We will not share the identity of participants in the study with anyone. The information we collect from this study will be kept confidential. Any information collected about your child will have a number on it instead of your child's name. Only the study team members will know what the number is and we will lock that information up.

We will share the knowledge that we get from the study with you before it is made available to the public. Confidential information will not be shared.

PART 2

Certificate of consent.

I have been invited to have my child participate in a study of artemether/lumefantrine used to treat malaria.

I have read the above information or it has been read and explained to me. I have had the opportunity to ask questions and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to my child's participation in this study.

Name of participant:-

_____

Name of parent or guardian:-

_____

Signature of parent or guardian:-

_____

Date:-_____

Witness' signature.

I have witnessed the accurate reading of the consent form to the potential participant's parent or guardian, who has had the opportunity to asked questions. I confirm that the participant's parent or guardian has given consent freely.

Name of witness:-_____

Signature of witness:-_____

Date:-_____

Investigator's signature:

I have accurately read or witnessed the accurate reading of the consent form to the potential participant's parent or guardian, who has had the opportunity to ask questions. I confirm that the participant's parent or guardian has given consent freely.

Name of investigator:-

_____

Signature of investigator:-_____

Date:-_____

Source: Adopted from WHO (2003) Assessment and Monitoring of Antimalarial Drug Efficacy for the Treatment of Uncomplicated P. falciparum Malaria

VERNACULAR VERSION OF INFORMED CONSENT

TAKARDAR NEMAN YARDA GUDANAR DA GWAJIN MAGANI

Sunan mai bincike:-

_____

Sunan maikatar mai bincike:-_____

Aikin da ake bincike akansa:-_____

KASHI NA DAYA

Sunana Muhammad Auwal. Dalibi ne ni a makarantar Ahmadu Bello ta Zaria. Ina aiki da Hukumar kula da asbitoci ta Jihar Kano.

Ina bincike ne akan yadda ake warkar da zazzabin cizon sauro da amfani da maganin zamani. Zazzabin cizon sauro ciwo ne mai hadari,amma ana warkar dashi da maganin zamani.

Dalilin binciken shine don a tabbatar da sahihancin maganin da ake kira artemether-lumefantrine wanda ake bayarwa kyauta a asibitocin Jihar Kano domin magance wannan cutar.

Muna neman yardar ku da amincewar ku don amfani da yaran ku yan kasa da shekara biyar da haihuwa dan gudanar da wannan bincike.

Haka kuma dammar ka ce ka shiga wannan binciken ko kada ka shiga.Rashin shigar dan ka ba zai shafi kulawa da yake samu a wannan asibiti ba . Kamar yadda aka saba , Zamu ba yaron ka magani na kwana uku wanda zai sha sau biyu arana, da safe da yamma.Wannan magani shine

hukumar kula da lafiya ta kasa ta yarda da ayi amfani das hi wajen magance zazabin cizon sauro . Sunan kamfanin da yake maganin da zamuyi amfani dashi shine IPCA Laboratories.

Wannan maganin yana da inganci kwarai wajen magance wannan cutar. Idan an sha maganin, akan dan ji jiri,da kasala, dad an tashin zuciya,amma wannan ba wata matsala bace.

Idan yakasance wajen yin bincike mungane cewa maganin baiwa yaron ka aiki ba,akwai wani magani na musamman da za ayi amfani das hi wajen magance wannan cutar data bijirewa wannan magani. Sanin cewa kwayar cutar tana bijirewa maganin shi yasa ake da bukatar lokaci- lokaci a dinga bincike akan sahihancin maganin.

Za a dauki jinin yaron ka don yin bincike ranar farko da ta biyu da ta uku,ajere.Daganan sai kuma bayan Duk sanda yaro yazo shan magani,ko gwajin ji , sai likita ya duba lafiyar sa kwarai.

Ana iya neman shawara wajen wanda aka aminta dashi akan wannan aiki. Za a gama wannan aiki bayan sati hudu. Zamu dauki cikakken adireshin ka da lambar waya dan saduwa da ku idan bukatar hakan ta taso.

Dukkan bayanin da bincike ya bamu zamu aji ye shi a matsayin sirri tsakanin mu da ku.

KASHI NA BIYU

YARDAR IYAYE AKAN GWAJIN MAGANI

Na gamsu da bayanin day a gabata akan yin bincike akan maganin zazzabin cizon sauro mai suna artemether-lumefantrine.

Na yarda batare da tursasawaba cewa da na ko ya ta ya//ta shiga wannan binciken

Sunan yaro/yarinya:-_____

Sunan iyaye ko mariki:-

_____

Sa hannun iyaye ko mariki:-_____

Kwanan wata:-_____

Sunan mai sheda:-_____

Sa hannu:-_____

Kwanan wata:-_____

Sunan mai bincike:-

_____

Sa hannun mai bincike da kwanan wata:-_____

Appendix IV.

CASE REPORT FORMS

Case report form follow up day 0	
Name of hospital: Town:: LGA State:	Study number Patient screening number Date of visit (dd-mm-yyyy).
Demographic data	
Date of Birth (dd-mmm-yyyy): or estimated age: in: Months ☐ years ☐ Height (cm) ☐ Weight (Kg) ☐ Sex: Male ☐ Female ☐	

Pre-treatment temperature
History of fever in previous 24hr? Yes ☐ No ☐ Temperature ^{0}C Axillary ☐ Tympanic ☐ Rectal ☐ Oral ☐
Thick blood smears for estimation of *Plasmodium falciparum* parasitic counts
Average number of asexual *Plasmodium falciparum* parasites/µl Presence of *P. falciparum* gametocytes? Yes☐ No☐ ((if yes not eligible) Were species other than *Plasmodium falciparum* present? Yes☐ No☐ if yes, which species? Yes ☐ No ☐
Prior medication
All prior medication, including natural remedies and homeopathic medicines, taken within the previous 14 days should be reported in this section. Has the patient taken any prior antimalairal medication? Yes☐ No ☐ if yes, please specify below, either the date of stopping or the 'ongoing' box should be checked

Medicine name (generic name)	Dates	Ongoing (Yes =)	Total daily dose and unit (e.g 400mg)	Route of administration	Indication for use
	Start: Stop:	☐			
	Start: Stop:	☐			
	Start: Stop:	☐			

Case report form: follow-up day 0 (page 2)				
Medication administration				
Names(s) of antimalarial drugs	Time of dose (hh:min)	Number of tables	Did the patient vomit	Time of vomiting
			Yes or no	
			Yes or no	
Name of other medicines				

Case report form: follow-up days 1,2,3,7,14,21 and 28
Study number Patient screening number Date of visit (dd-mm-yyyy).
Clinical status
Presence of danger signs of severe or complicated malarial? Yes ☐ No ☐ if yes, perform thick blood smear Temperatures: ^{0}C Axillary ☐ Tympanic ☐ Rectal ☐ Oral ☐
Thick blood smears for estimation of *Plasmodium falciparum* parasite counts
Average number of asexual *Plasmodium falciparum* parasites/µl Presence of *P. falciparum* gametocytes? Yes ☐ No ☐ Were species other than *Plasmodium falciparum* present? Yes ☐ No ☐ If yes, which species? *P.virax* ☐ *P. ovale* ☐ *P.malariae* ☐
adverse events
presence of an adverse event? Yes ☐ No ☐ If yes, name the adverse event It is a serious adverse event? Yes ☐ No ☐
Medication administration

Names(s) antimalarial drug	Time of dose (hh:min)	Number of tables	Did the patient vomits yes or no	Time of vomiting
Name of Antimalarial				

Source: Adopted from WHO (2003). Assessment and Monitoring of Antimalarial Drug Efficacy for the Treatment of Uncomplicated P. falciparum Malaria

Appendix V

SCHEDULE OF FOLLOW-UP ACTIVITIES

Day

Procedure								
	0	1	2	3	7	14	21	28
Clinical assessment	X	X	X	X	X	X	X	X
Temperature	X	X	X	X	X	X	X	X
Blood slide for parasite count	X		X	X	X	X	X	X
Blood for:								
Hemoglobin or haematocrit	(X)					(X)		(X)
Treatment								
Medicine to be test	X	X	X					
Rescue treatment		(X)	(X)	(X)	(X)	(X)	(X)	(X)

Parentheses denote conditional or optional activities. For example, treatment would be given on days 1 and 2 only for 3-day dosing. On day 1, the patient should be examined for parasitaemia if he or she has any danger signs. Rescue treatment could be given on any day, provided that the patient meets the criteria for treatment failure. Extra days are any days other than regularly scheduled follow-up days when the patient returns to the facility because of recurrence of symptoms. On extra days, blood slides may be taken routinely or at the request of the clinical staff

Source: Adopted from WHO (2009). Methods of Surveillance of Antimalarial Drug efficacy

Appendix VI

SERIOUS ADVERSE EVENT REPORT FORM

<table>
<tr><td colspan="2">Serious adverse event report form</td></tr>
<tr><td>Name of hospital

Town:

LGA:

State</td><td>Study number

Patient identity number

Date of visit (dd-mm-yyyy).

Follow-up day:</td></tr>
<tr><td colspan="2">Demographic data</td></tr>
<tr><td colspan="2">Date of Birth (dd-mmm-yyyy): or estimated age: in: Months ☐ years ☐

Height (cm) ☐ Weight (Kg) ☐

Sex: Male ☐ Female ☐</td></tr>
<tr><td colspan="2">Serious adverse event</td></tr>
<tr><td colspan="2">☐ death

☐ life-threatening

☐ hospitalization or prolongation of hospitalization

☐ permanent disability

☐ congenital anomaly or birth defect

date of occurrence (dd-mmm-yyyy):</td></tr>
<tr><td colspan="2">Describe the serious adverse event (included all relevant laboratory results):</td></tr>
<tr><td colspan="2">Describe how the reaction was treated</td></tr>
</table>

Serious adverse event report for (page 2)

Comments (e.g. relevant medical history, drug allergies, previous exposure to similar drugs laboratory data, whether reaction abated after stopping the drug whether reaction reappeared after reintroduction):

Outcome

☐ Recovered completely

☐ Not yet recovered

☐ Recovered with long-term consequences

If patient recovered, provide date of recovery (dd-mmm-yyyy):

Medicines (list the medicine suspected of causing the serious adverse event as well as all concomitant medicines)

Brand name, batch number, manufacturer name (list suspected medicine first)	Daily dose	Route	Start date	End date	Indications for use

Reporting Researcher

Name:

Qualification:

Address;

Phone:

Signature Date:

Source: Adopted from WHO (2003). Assessment and Monitoring of Antimalarial Drug Efficacy for The treatment of Uncomplicated P. falciparum Malaria

Appendix VII

PROTOCOL USED FOR STUDYING ARTEMETHER/LUMEFANTRINE EFFICACY AND SAFETY IN FALCIPARUM MALARIA IN CHILDREN UNDER 5 YEARS OF AGE.

Day 0

Screening

- Clinical assessment, including measurement of weight and height; referral in cases of severe malaria or danger signs;
- Measurement of temperature;
- Parasitological assessment;
- Informed consent

Enrolment

- Treatment, first dose;

Optional

- Hemoglobin/Haematocrit

Day 1

- Clinical assessment, referral in case of severe malaria or danger sign;
- Measurement of axillary temperature;
- Parasitological assessment in cases of severe malaria or danger signs;
- Treatment, second dose or alternative treatment in case of early treatment failure

Day 2

- Clinical assessment; referral in cases of severe malaria or danger signs;
- Measurement of axillary temperatures;
- Parasitological assessment;
- Treatment, third dose or alternative treatment in case of early treatment failure

Day 3, day 7, day 14, day 21, day 28.

Clinical assessment; referral in cases of severe malaria or danger signs;

Source: Adopted from WHO (2003). Assessment and Monitoring of Antimalarial drug Efficacy for the Treatment of Uncomplicated P. falciparum Malaria