PARASITAEMIA CLEARANCE AND PHARMACOKINETICS OF ARTEMETHER/ LUMEFANTRINE CO-ADMINISTERED WITH UNSWEETENED NATURAL COCOA POWDER

A THESIS SUBMITTED BY

AKOTUAH PRINCE APPIAH

(10383177)

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DECLARATION

BY CANDIDATE

This thesis is submitted to the University of Ghana (UG), School of Graduate Studies through the College of Health Sciences, Medical School, Department of Pharmacology. This study is entirely my own research, which was carried out under the supervision of the undersigned supervisors in the Department of Pharmacology. I hereby declare that, except for references to other's research works, which have been duly acknowledged. This thesis has not been submitted to any other academic institution for the purpose of obtaining any academic award.

STUDENT: AKOTUAH PRINCE APPIAH (10383177)

Signature.. Date: 09/09/2022

SUPERVISORS

Signature… Date: 09/09/2022

PROF ISAAC J. ASIEDU-GYEKYE

Signature… Date: 9th September, 2022.

DR PHILIP DEBRAH

DEDICATION

I humbly dedicate my work to God Almighty, in memory of my late father Mr. Frank Anim Akotuah and to my lovely mother Mrs. Juliana Akotuah and wonderful siblings for their moral, financial, spiritual support and encouragement. God richly bless you.

ACKNOWLEDGEMENTS

Firstly, I want to say a big thank you to God for the strength to carry through this academic journey. My profound gratitude to my supervisors Prof. I.J Asiedu-Gyekye, Dean of University of Ghana School of Pharmacy and Dr Phillip Debrah, Department of Pharmaceutics and Microbiology- University of Ghana School of Pharmacy for their wisdom, guidance and unflinching support.

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LIST OF ACCRONYMS AND ABBREVIATIONS

RBCs – Red Blood Cells

WHO - World Health Organization

A/L - Artemether/ Lumefantrine

UNCP - Unsweetened Natural Cocoa Powder

DDT - Dichloro Diphenyl Trichloroethane

WWARN - World Antimalarial Resistance Network

GI – Gastro Intestinal

API – Active Pharmaceutical Ingredient

IPCA – Indian Pharmaceutical Combined Association

LFT – Liver Function Test

AST – Aspartate Amino Transferase

ALT – Alanine Amino Transferase

ALP – Alkaline Phosphatase

HPLC – High Performance Liquid Chromatography

SD – Sprague Dawley

XO- Xanthine Oxidase

LLITNs – Long-Lasting Insecticide Treated Nets

IRS- Indoor Residual Spraying

DHFR - Dihydrofolate Reductase

TNF- Tumour Necrosis Factor

ICAM - Intracellular Adhesion Molecule

RDT – Rapid Diagnostic Test

GHS – Ghana Health Service

LOX – Lipoxygenase

COX – Cyclooxygenase

A/A - Artesunate/Amodiaquine

D/P - Dihydroartemisinin/Piperaquine

ABSTRACT

Background: One major setback of the use of Artemether/Lumefantrine (A/L) is its high dependence on fatty diets for maximum gastrointestinal (GI) absorption. This is further compounded by nausea and lack of appetite by malaria-infected patients posing a risk of treatment failure and poor therapeutic outcomes. The antimalarial activity and fat content in Unsweetened Natural Cocoa Powder (UNCP) may produce synergism when taken as a dietary supplement with A/L in the efficient treatment of uncomplicated malaria.

Aim: To evaluate the effect of UNCP on plasma concentration and malaria parasite clearance of A/L co-administration in murine models.

Methodology: An antimalarial experimental study was carried out in A/L-sensitive *Plasmodium berghei*-infected male Sprague-Dawley (SD) rats to examine the effect of UNCP (300, 600, 900, 1,200 and 1,500 mg/kg), n = 5, when administered alone and in combination with A/L. Also, a pharmacokinetic study was performed on healthy non-malarious male SD rats co-administered A/L with varying doses of UNCP (300, 600, 900, 1,200 and 1,500 mg/kg), n = 3, to assess the effect of UNCP on the plasma drug concentration, bioavailability and other pharmacokinetic parameters using HPLC/UV-Vis method. This was followed by a Liver Function Test (LFT) on the serum samples of the various groups. Phytochemical screening was performed on UNCP to determine the various phytochemicals present. A total fat assay was also performed using the Soxhlet solvent extraction method to ascertain the percentage fat content of UNCP.

Results

Results indicate a significant difference in parasite clearance for A/L+UNCP (1,200 and 1,500 mg/kg) groups at 36 and 48 hours of administration. A/L + UNCP (1,500 mg/kg) group recorded the most significant parasite decrease ($F_{6,28}$=1333, $P < 0.0001$) in comparison with Coartem® only group (positive control) with a percentage drop of 55.89% and 39.63% respectively and this was followed by A/L + UNCP (1,200 mg/kg) ($F_{6,28}$=1333, $P < 0.0210$) with a percentage drop of 51.98% at 36 hours. A similar trend was observed at 48 hours for A/L + UNCP (1,500 mg/kg) ($F_{6,28}$=1333, $P < 0.0017$) and A/L + UNCP (1,200 mg/kg) groups ($F_{6,28}$=1333, $P < 0.0391$) with percentage drop of 71.97% and 71.3% respectively in comparison with Coartem® only group, (percentage drop of 57.68%) at 48 hours. The UNCP only groups however did not show significant antimalarial activity, but results showed that 1,200 mg/kg and 1,500 mg/kg UNCP only groups survived 24 hours longer than the other UNCP only groups with their survival rate expressed as Mean Survival Time (MST); 2 ± 0.4 days/animals and 2 ± 0.8 days/animals respectively. On the other hand, there was no significant difference in MST for the A/L+UNCP groups (28 ± 0.6 days/animals).

Pharmacokinetic studies indicated a significant difference in the area under the curve ($AUC_{0\rightarrow24}$) over the 24 hours post drug administration of Lumefantrine for A/L+UNCP (1,200 mg/kg) and A/L+UNCP (1,500 mg/kg) groups (**$P < 0.0091$) and (***$P < 0.0003$) in comparison with the Coartem® only group respectively. Similarly, there was a significant difference in peak serum concentration (Cmax) for A/L+UNCP (1,200 mg/kg) (**$P < 0.0051$) and A/L+UNCP (1,500 mg/kg) (***$P < 0.0003$) groups in comparison with the Coartem® only group. However, there was no significant difference for (half-life) $t_{1/2}$, (elimination rate constant) K_e and (peak time) Tmax amongst the various groups. Phytochemical analysis showed the presence of alkaloids,

saponins, tannins, glycosides, flavonoids, phenols and the absence of reducing sugars and terpenoids with a percentage fat content of 28.11%. Also, LFT results did not show any significant difference amongst the various groups.

Conclusion

Higher doses of UNCP (1,200 and 1,500 mg/kg) co-administered with A/L showed significant antimalarial activity and a higher plasma concentration of A/L with a safe hepatic profile.

CHAPTER 1

GENERAL INTRODUCTION

Background

Malaria is a disease caused by a group of protozoan parasites under the genus Plasmodium transmitted by the bite of an infected female anopheles mosquito. There are more than hundred species of plasmodia that can infect animal species but only five in humans, namely; *Plasmodium ovale, P. falciparum, P. malariae, P. vivax and P. knowlesi* which is the latest amongst the five and has been reported to be the cause of zoonotic malaria in humans which is although rare but highly fatal (CDC, 2019). Amongst these, *P. falciparum* and *P. vivax* pose the greatest threat to humans, however, in the African sub regions, *P. falciparum* remains the main culprit accounting for about 99.7% of estimated malaria cases, whereas *P. vivax* is most predominant (74%) in the North and South Americas (WHO, 2019).

Malaria remains an important cause of illness and death across the globe, according to the world malaria report by the WHO, an estimated 219 million cases (95% confidence interval) of malaria occurred worldwide with estimated deaths of about 435,000 of which about 93% occurred in sub-Saharan Africa. Also, children under 5 remain the most vulnerable age group and accounted for about 60% of malaria deaths worldwide. Despite the high numbers, the global fight against malaria has made great strides worth mentioning.

The malaria parasite has a complex, multistage life cycle occurring within two living organisms, the vector mosquitoes and the vertebrate hosts (Bozdech et al., 2003; Catteruccia, 2007). Mosquitoes are the definitive hosts, wherein the sexual phase of the parasite's life cycle occurs, whiles humans serve as the intermediate host for the asexual stage of the parasite (Njabo et al., 2011; Reddy et al., 2011).

In humans, there are both the erythrocytic and exo-erythrocytic stages, with the former being the stage where there is the manifestation of clinical symptoms (Kamareddine, 2012; Ménard et al., 2016).

The fight against malaria employs a multifaceted approach, involving vector control interventions such as the use of Insecticide-Treated Nets (ITNs), Indoor Residual Spraying (IRS) and also the therapeutic approach which involves the clinical diagnosis and treatment with chemotherapeutic agents (N'Guessan, Corbel, Akogbéto, & Rowland, 2007; Russell et al., 2011).

The standard approach in the management of malaria worldwide is the use of the Artemisinin-based Combination Therapy (ACT) (Trape et al., 2011; WHO, 2019). In Ghana, the three main ACTs used in treating uncomplicated malaria include, Artemether/Lumefantrine (A/L), Artesunate/Amodiaquine (A/A) and Dihydroartemisinin/Piperaquine (D/P) which are given based on a patient's body weight (Dodoo et al., 2009; GHS, 2019).

Unsweetened Natural Cocoa Powder (UNCP) is a popular nutraceutical obtained from *Theobroma cacao* (cocoa) and has been found to possess in vitro anti-plasmodial activity (Amponsah et al., 2012). Additionally, it mitigates A/L induced cardiotoxicities, hepatoxicities and nephrotoxicities investigated in animals (Asiedu-Gyekye et al., 2016). Also, UNCP may contain enough fat to aid the absorption of A/L. These properties suggest the possibility of a synergism when it is combined with A/L in the management of uncomplicated malaria.

Problem Statement

Amongst the three main ACTs approved by the WHO in the management of uncomplicated malaria in Ghana, A/L is the most commonly used (GHS, 2019). However, poor gastrointestinal (GI) absorption of A/L presents a major challenge. This is because it is highly dependent on dietary

fat for GI absorption. This is more pronounced in Lumefantrine than Artemether with the former resulting in less than 10% absorption in the absence of food (Ashley et al., 2007). Studies have shown a two- and sixteen-fold increase in systemic bioavailability of Artemether and Lumefantrine respectively when taken with fatty diets (Ashley et al., 2007). Notwithstanding, these figures are difficult to achieve in malaria patients since they usually present with nausea and loss of appetite hence are unable to take the drug with food. Therapeutic outcomes may become quite a challenge in the future since it is largely dependent on the oral bioavailability of A/L to ensure efficient parasite clearance. On the other hand, UNCP which is a common beverage in Ghana has been found to possess an in vitro antimalarial activity (Amponsah et al., 2012) and also attenuates A/L-induced organ toxicities investigated in animals (Asiedu-Gyekye et al., 2016). Additionally, it is known to contain palmitic acid, stearic acid and oleic acid (Ortega et al., 2009) and these fatty acid components may aid the absorption of A/L. These qualities make UNCP a great candidate in combination with A/L.

Justification of the Study

Malaria still remains a major health challenge especially in Africa. The WHO reports a total of 219 million estimated cases with 435,000 deaths of which 93% of the deaths occurred within sub-Saharan Africa which includes Ghana. Children under 5 years and pregnant women remain the most vulnerable groups accounting for about 60% of total malaria deaths. A/L is one of the commonly used antimalarials in Ghana, however, its poor GI absorption presents a major challenge because it is highly dependent on dietary fat for optimum GI absorption. Most malaria patients tend to experience nausea and loss of appetite resulting in poor treatment outcomes due to reduced GI absorption of A/L. Cocoa is one of the major crops produced in West Africa. UNCP, a product of cocoa has been found to possess antimalarial activity and also may contain enough fat to aid

absorption of A/L for a more efficient parasite clearance. Ironically, even though West Africa accounts for about 70% of cocoa produced worldwide, it still remains one of the malaria-endemic regions with little benefit being drawn from this crop in the fight against malaria. Thus, this research seeks to explore the potentials of UNCP as possible combination with A/L in efficient management of uncomplicated malaria.

Research Hypothesis

A/L+UNCP coadministration will produce a higher plasma concentration and better parasite clearance than UNCP or AL alone.

Aim of Study

To evaluate the effect of UNCP on plasma concentration and malaria parasite clearance of A/L co-administration in murine models.

Specific Objectives

- To perform phytochemical analysis and determine the percentage fat content in UNCP
- To evaluate the extent of parasite clearance when A/L is co-administered with UNCP
- To evaluate the bioavailability of A/L when co-administered with UNCP using some pharmacokinetic parameters such as Cmax, AUC, Tmax, etc.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 MALARIA BACKGROUND AND EPIDEMIOLOGY

According to the WHO, malaria is a disease caused by the infection of the red blood cells by protozoan parasites of the genus Plasmodium and it is transmitted by the bite of an infected female anopheles mosquito. There are five main types of the plasmodium parasites responsible for the disease in man, and these are *Plasmodium vivax, Plasmodium ovale, Plasmodium knowlesi, Plasmodium malariae, Plasmodium falciparum*. Amongst these *P. vivax* and *P. falciparum* are the most common and pose the greatest threat to humans (CDC, 2019). The 2019 WHO malaria report indicates estimated malaria deaths of 405,000 globally with children under 5 years accounting for 67% of these deaths, amongst the vulnerable groups include pregnant women with prevalence of about 11 million leading to low birth weight children of about 872,000 with West Africa having the highest low birth weight prevalence. Also, Africa accounts for 94% of all malaria deaths of which countries such as Nigeria, DR Congo, Tanzania, Angola, Mozambique and Niger collectively contributed about 52% of these deaths.

These figures are alarming even though there has been a global decline which reflects the crucial effort by the WHO. The rate of decline however, has been extremely slow especially in Africa. For instance, 19 sub-Saharan countries and India accounted for about 85% of the global malaria burden. Also, of the 10 highest burdened countries in Africa, Ghana and Nigeria reported the highest absolute increases in malaria cases in 2019 compared to the previous year. Thus, it is obvious that a lot of effort is needed in the fight against malaria in our bid to find comprehensive solution.

2.2 MODE OF TRANSMISSION OF MALARIA

The plasmodium parasite has two hosts; the female anopheles mosquito and humans with the latter serving as an intermediate host. Malaria is a vector-borne disease and it is introduced by the bite of an infected female anopheles mosquito (CDC, 2019). After a blood meal, an inoculum of the plasmodium parasite is released into the host, it attacks the liver cells then consequently invades the red blood cells, multiplies and infects more red blood cells resulting in their degradation and depletion, with fever, general body pains and anaemia being the cardinal signs and symptoms (Dambhare, Nimgade, & Dudhe, 2012; Tahita et al., 2013). Although transmission by mosquito bite forms the majority, there are other modes such as blood transfusion by an infected person, the use of contaminated needles and via pregnant mother to foetus (Traoré, Sangaré, & Traoré, 2017).

2.2.1 The Anopheles Mosquito

Anopheles refers to a genus of mosquito consisting of over 400 species, however only 30-40 can transmit the plasmodium parasite which causes malaria. Amongst them, *Anopheles gambiae* remains the most predominant species in transmitting *P. falciparum* in humans due to factors such as parasite-host compatibility and decreased or lack of mosquito's immune response against invading plasmodium parasites (Moreno et al., 2010; Sinka et al., 2010).

Just like most insects, the life cycle of the anophelines consist of the egg, larvae, pupa and adult stages, with the first three phases altogether occurring within 5-14 days depending on the favorability of the ambience such as temperature (18 - 32°C), humidity (>60%) and the species (Beck-Johnson et al., 2013; Imbahale et al., 2011; Murdock, Blanford, Luckhart, & Thomas, 2014). The adult female, *A. gambiae* can feed on nectar and other sugar sources to provide energy and blood which contains the necessary proteins needed for egg development after mating.

Research has shown the behavioral tendency of plasmodium-infected mosquitoes to be attracted to the odour and breath of humans than the uninfected mosquitoes. It posits that the infected mosquitoes are three times as likely as uninfected ones to respond to human smells and this influence by the parasites is to ensure their survival and continuity in their hosts (LSHTM, 2013). Apart from the Anopheles mosquito, there are other genera of mosquitoes such as Aedes, Culex, Funestus, Culiseta, etc. who serve as vectors for diseases other than malaria.

2.2.2 The Plasmodium Parasite

Plasmodium refers to a genus of unicellular eukaryotes of the phylum Apicomplexa that undergo asexual and sexual reproduction in a vertebrate and insect host respectively. Generally, mosquito serves as the blood-sucking vector/host although other insects such as the sand-fly can also transmit the parasite in reptiles (Busula, Verhulst, Bousema, Takken, & de Boer, 2017; Mitchell & Catteruccia, 2017). In the vertebrate host, there are a broad array of organisms such as birds, reptiles and mammals infected by more than 200 species of the plasmodia with only five primarily implicated in humans (Stanczyk, Mescher, & De Moraes, 2017). *P. falciparum* is the most prevalent in WHO African region (99.7%), South-East Asia(50%), Eastern Mediterranean region (71%) and Western Pacific region (65%) (WHO, 2019). Globally, 53% of *P. vivax* burden occurs in the WHO South-East Asia region, with India accounting for the majority (47%), it is also the predominant malaria parasite in the Americas region representing about 75% of malaria cases (WHO, 2019). In Ghana, *P. falciparum* accounts for about 97.4% of all malaria cases, followed by *P. malariae* (1.1%) *and P. ovale* (0.8%) (GHS, 2019).

2.2.2.1 *Plasmodium Malariae*

Although these species share a lot of common attributes, there are still a few distinct characteristics; for example *P. malariae* has a unique feature of being the only malaria parasite with fevers occurring every 72 hours (Quartan fever) as compared with the other species which occur every 48 hours (Tertian fever) (Lalremruata et al., 2015). Also, it usually has lower parasite counts than in patients infected with other species which could be due to low number of merozoites produced during the pre-erythrocytic stage, the preference of the parasite to older red blood cells, thus these factors result in earlier development of immunity by the host (CDC, 2019). Another defining feature that has been discovered in clinical presentations by patients is the presence of edema and nephrotic syndrome. It is however noted that due to the similarities in appearance in pathogens, infections caused by *P. knowlesi* is often misdiagnosed as *P. malariae,* hence molecular analysis is needed to detect the exact pathogen (Bruce, Macheso, Galinski, & Barnwell, 2006; Collins & Jeffery, 2007).

2.2.2.2 *Plasmodium Vivax and Ovale*

The distribution of *P. ovale* is concentrated in sub-Saharan Africa and the Western Pacific Islands, reports indicate about 15 million infection cases yearly (Collins & Jeffery, 2005). There are two *P. ovale* sub-species that have been identified and classified; *P. ovale curtisi and P. ovale wallikeri,* of which both exhibit tertian fever in humans (Sutherland et al., 2010).

For *P. vivax,* most literature attribute its disease burden to the absence of the Duffy Antigen Receptor Chemokine (DARC) which is usually absent especially in Africans (Langhi & Orlando Bordin, 2006). The DARC serves as a receptor specifically for *P. vivax and P. knowlesi* parasites.

Hence most *P. vivax* infections occur usually in Asia, Latin America and some few parts of Africa with an estimated number of 2.5 billion people at risk to infection (Gething et al., 2012). Current reports indicate the exhibition of atypical symptoms of *P. vivax* and *P. vivax*-infected patients such as hiccups, absence of fever and pain while swallowing (Francisco, 2019).

Morphologically, it is quite difficult to distinguish between *P. ovale* and *P. vivax* under microscopic examination especially if the parasite count is low and this is due to the similar structures, they both possess. There is the presence of Schuffner's dots on the surface of parasitized red blood cells (RBCs) and these are larger and darker in *P. ovale* than in *P. vivax*. Also, both species have their infected RBCs being enlarged and same fever periodicity (48 hours) (Obare et al., 2013). However, one way of differentiating these species morphologically is by examination of their mature schizonts. *P. vivax* usually has more than twelve nuclei within its schizont whiles is less in *P. ovale* (WHO, 2019).

2.2.2.3 *Plasmodium Knowlesi*

Of all the *plasmodium* species which cause malaria in humans, the most recently discovered is *P. knowlesi* with its unique ability of being transferred from primates to humans (Pasini, Zeeman, Voorberg-Van Der Wel, & Kocken, 2018). Like all the other *Plasmodium* species, it shares its life cycle in both mosquitoes and warm - blooded hosts (humans and apes). It is common in South East of Asia where it infects macaque monkeys with *Anopheles hacker* and *Anopheles latens* being the mosquito vectors of transmission, especially in Malaysia where it is the main culprit of malaria infections (Butcher & Mitchell, 2018; Garrido-Cardenas, González-Cerón, Manzano-Agugliaro, & Mesa-Valle, 2019). Due to the uniqueness of these Anopheles species as specific vectors for *P. knowlesi* in these areas, thus it is restricted to that region only (Pasini et al., 2018).

2.3 PLASMODIUM FALCIPARUM

Currently, in terms of all the five Plasmodium species causing malaria in humans, *P. falciparum* remains the most virulent and deadly, accounting for about 91% of all malaria cases worldwide (Perkins et al., 2011; WHO, 2019).

2.3.1 Life Cycle of The *Plasmodium falciparum* Parasite

The life cycle of the *P. falciparum* consists of the sexual phase which occurs in mosquitoes serving as definitive host and the asexual phase in human. In the mid gut of the female anopheline mosquito, the flagellated microgametes of the male gametocyte fertilize the female gametocyte to form a zygote which then develops into ookinete. This traverses the mid gut epithelium and settles at the basal lamina as oocyst where it undergoes several divisions, migrates to the salivary gland and matures into sporozoites (Gerald, Mahajan, & Kumar, 2011).

2.3.2 The Exo-erythrocytic Stage

During a blood meal by the malaria-infected mosquito, it releases sporozoites into the human host with each infective bite containing about 20 - 200 sporozoites (Garcia, Puentes, & Patarroyo, 2006). Although most of the sporozoites may be cleared by the host immune system within half an hour, the few that elude the immune cells infect the liver (Gerald et al., 2011). Research indicates that the characteristic possession of actomyosin aids the motility and invasion of the sporozoites in the host organism (Kappe, Buscaglia, Bergman, Coppens, & Nussenzweig, 2004). In the liver cells, the parasite takes on a structure known as parasitophous vacuole (PV) which protects it from phagolysosomes of the host cells (Laliberte, Carruthers, & sciences, 2008). The sporozoites then undergo 13-14 rounds of mitosis and meiosis to become schizonts which

mature into merozoites which are finally offloaded into the blood stream in vesicles called merosomes (Sturm, Amino, & et al., 2006). The liver of the host can produce as many as 90,000 merozoites (Vaughan & Kappe, 2017).

2.3.3 The Erythrocytic Stage

The merozoite infect the RBCs with the help of its invasion organelles made up of apical complex, pellicle and surface protein and there are three distinct phases of invasion namely pre-invasion, invasion and ethinocytosis. These occur within a minute and half after their release from the liver (Gilson & Crabb, 2009). *P. falciparum* use several receptors for cellular engagement including Glycophorin, the major glycoprotein on the surface membrane of RBCs and Erythrocyte Binding Protein (EBA-140) which specifically binds to Glycophorin C on RBCs (Pasvol, 2003).

Inside the erythrocyte, there is transport and digestion of haemoglobin into haem and globin. The haem is detoxified by polymerization and sequestration as inert crystalline deposit known as hemozoin, while the amino acid in the globin portion is utilized for protein synthesis by the organism (Elliott et al., 2008).

The first form the parasite exhibited in the RBCs is the ring form known as trophozoites which mature into schizonts rapturing and releasing merozoites. The *P. falciparum* has the shortest incubation time of about 11 days. However, some of the parasites develop into sexual erythrocytic known as gametocytes. The microgametocytes (male) and macrogametocytes (females) are ingested by an anopheles mosquito during a blood meal. The parasites multiplication in the mosquito is known as the sporogonic cycle (Trampuz, Jereb, Muzlovic, & Prabhu, 2003). While in the mosquito's stomach, the microgametes penetrate the macrogametes to form generating zygotes. The zygotes are then turn into motile and elongated forms known as ookinetes which

invade the midgut wall of the mosquito where they develop into oocysts. The oocysts then grow, rupture and release sporozoites, which make their way into the mosquito's salivary glands (CDC, 2019).

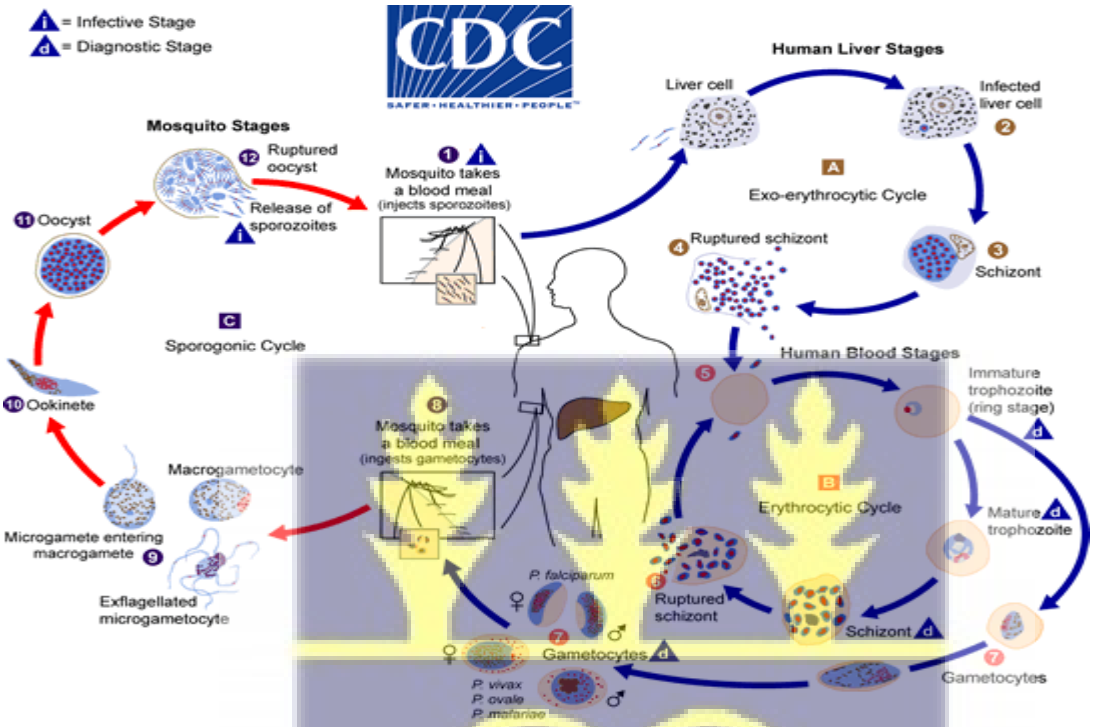


Figure 2.1: Life cycle of the Plasmodium parasite

(Retrieved from https://www.cdc.gov/malaria/about/biology/index.html)

2.4 MANAGEMENT OF MALARIA

2.4.1 Laboratory Investigations

2.4.1.1Rapid Diagnostic Test

Malaria parasite presence identification is a very key component in the entire clinical diagnosis process. The two common methods include the use of Rapid Diagnostic Test (RDT) kits and microscopy (WHO, 2019). The RDT involves the qualitative detection of parasite antigens which are produced by the plasmodium species. There are three main types of RDTs antigens, namely Histidine-rich Protein 2 (HrP2), Plasmodium Lactate Dehydrogenase and Aldolase (Murray & Bennett, 2009). Amongst these, the most commonly used in Ghana is HrP2 test kit (GHS, 2019). Over the past five years, RDT has been very helpful especially in rural parts of the world in the diagnosis of malaria. This is because it is cheaper, more accessible and requires less expertise in comparison with microscopy (Ahorlu, Koram, Seake-Kwawu, & Weiss, 2011; Baiden et al., 2014). However it has key limitations such as inability to determine parasite density, parasite growth stage and differentiate and identify the various species (Ayi et al., 2010).

2.4.1.2 Microscopy

The use of microscopy in malaria parasite detection still remains the gold standard for a number of reasons such as; ensuring more accurate results in the quantification of parasites and its ability to detect the various parasite species including their various growth forms (McMorrow, Aidoo, Kachur, & Infection, 2011). Although microscopy is highly effective, it is not ideal in a small community health setup due to the need for training and expertise, cost in purchase and maintenance of microscope, etc. (Ayi et al., 2010; Baiden et al., 2014).

There are two main microscopic techniques used in malaria parasite detection namely; thick and thin blood film which are both prepared with the help of giemsa stain.

2.4.1.3 Giemsa Stain

Giemsa stain is a solution mixture composed of methylene blue and eosin. The methylene blue stains parasite cytoplasm blue, while the eosin stains parasitic chromatin red or pink. It is the most common stain used in malaria laboratory examinations. There are two main Giemsa stain preparations; the rapid (10% Giemsa solution) which is commonly used and the slow method (3% Giemsa) (Barcia, 2007; Iqbal et al., 2003).

2.4.1.4 Thick Blood Film

The thick blood film consists of several layers of numerous RBCs and WBCs concentrated on a glass slide surface. It is the recommended technique in the identification of the presence of malaria parasite and ideal in the detection of samples with low parasite density due to its high sensitivity (Bejon et al., 2006).

2.4.1.5 Preparation of Thick Blood Film

A circular film of about 1cm in diameter is made with the corner of the spreader and allowed to dry. It is then dipped into a beaker containing distilled water and removed immediately and allowed to dry- this process is performed to dehaemoglobinize the RBCs in the blood. The smear is stained with 10% Giemsa stain for about 10 – 20 minutes then washed of gently using buffered water, allowed to dry and examined under a light microscope (WHO, 2010).

2.4.1.6 Examination of Thick Blood film

The number of parasites seen on one tally counter and the number of white blood cells on the oil of the immersion field is counted. When counting, the number of parasites relative to the number of leukocytes is calculated and expressed as parasites per microlitre of blood from the simple mathematical formula below:

$$\text{Number of parasitized RBCs per } \mu L = \frac{\text{Number of parasitized RBCs in 10 fields}}{\text{Number of WBCs 10 fields}} \text{ X 8000 WBCs ... (1)}$$

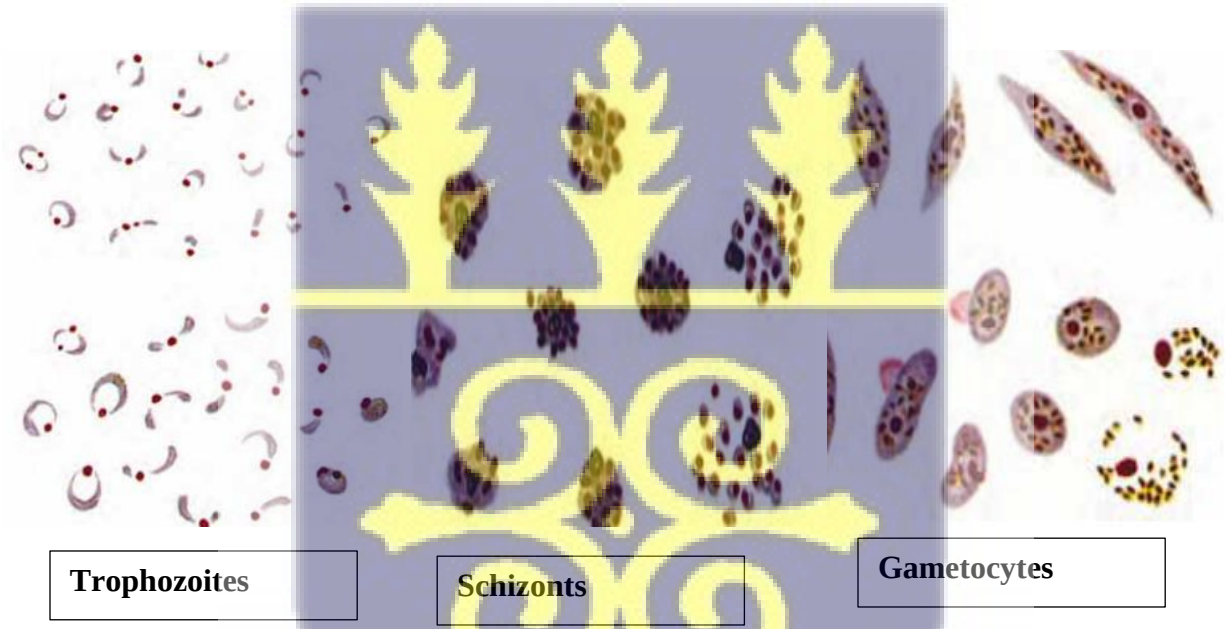


Figure 2.2: Thick film preparation of reproductive forms of *P. falciparum* under light microscope (Retrieved from WHO Microscopy Learner's Guide Manual)

2.4.1.7 Preparation of Thin Blood Film

The collected blood drops on the glass slides is spread with another clean slide as a spreader. This is done by allowing the blood to run along the edge of the spreader. The spreader is then firmly pushed along the slide at an angle of 45° with even contact on the surface of the other slide while the blood is being spread. The blood is allowed to dry for about 2 minutes and later fixed with methanol. The smear is stained with 10% Giemsa stain, and examined under a light microscope.

2.4.1.8 Examination of thin blood film

The slide is placed on a stage sitting the 100x objective lens and a drop of immersion oil applied at the middle of the thin film. The mechanical stage is racked up until the objective lens touches the immersion oil. The blood film is then examined along the edge of the film and then moving the slide inwards for one field up to 10 fields (WHO, 2010). The number of parasitized red blood cells will be divided by the total number of red blood cells and then multiplied by a hundred to calculate the % parasitaemia as shown in equation (2) below.

$$\text{Percentage parasitemia} = \frac{\text{Number of parasitized RBCs}}{\text{Total number of RBCs}} \text{ X } 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

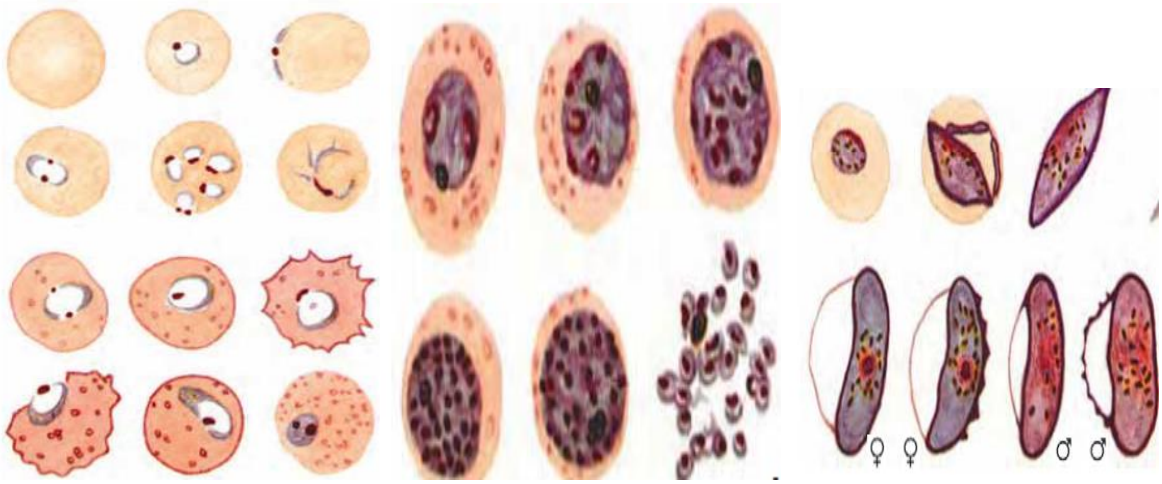


Figure 2.3: Thin film preparation of reproductive forms of *P. falciparum* under light microscope

(Retrieved from WHO Microscopy Learner's Guide Manual)

2.4.1.9 Polymerase Chain Reaction

The use of Polymerase Chain Reaction (PCR) is one of the most modern molecular biology approaches in malaria parasite detection. It holds several advantages over the above mentioned especially in scenarios where parasite morphology in differentiation of species is unreliable; this is because this method relies purely on the genetic composition which is unique to every species. It is highly sensitive and specific such that a fraction of genetic material present in a large volume of sample can be amplified to help detect parasite in very low concentration samples, also, it can help differentiate the various species of malaria parasites present in a microlitre of blood. Some of the common PCR methods available include Nested PCR and Multiplex PCR (Moody, 2002).

It is however worth noting that even though PCR possesses enhanced sensitivity and specificity considering the time, resources and expertise needed in parasite detection especially in acute cases

does not make it an ideal candidate currently, hence only useful for strain variations, mutations and drug-resistant gene studies (Gama et al., 2007).

2.4.2 CLINICAL INVESTIGATIONS

The WHO clinically classifies malaria into two main forms, uncomplicated and complicated/severe malaria. The key difference is the dysfunction and damage to vital organs which occurs in severe malaria but is absent in uncomplicated malaria, hence different treatment approaches are recommended (WHO, 2019).

2.4.2.1 Uncomplicated Malaria

According the WHO, malaria diagnosis standards, uncomplicated malaria is diagnosed by the presence of fever or recent history of fever with the absence of vital organ damage and a positive laboratory test for malaria (WHO, 2019). Other symptoms include headache, nausea/vomiting, loss of appetite, sweating, abdominal pains and bitter taste(Bartoloni, Zammarchi, & diseases, 2012; Silva-Nunes & Ferreira, 2007).

2.4.2.2 Severe Malaria

It is defined as a confirmed malaria with any clinical or laboratory evidence of dysfunction of vital organs. Even though it affects all ages, it is more frequent in high risk groups such as; children under five years, pregnant women, non-immune and immunosuppressed patients (GHS, 2019). Usually the delay in diagnosis of uncomplicated malaria especially in infants results in the rapid development of severe malaria (WHO, 2019). The mortality of untreated severe malaria (especially cerebral malaria) is 100%, however, with prompt and effective treatment and

supportive care, the rate falls to about 10-20%. The global annual incidence is approximately 2 million cases, with about 90% affecting children under five.

The pathogenesis of cerebral malaria involves a lot of immune and inflammatory markers such as Intracellular Adhesion Molecule (ICAM-1), Tumour Necrosis Factor (TNF) and Interleukin-10 (IL-10) whose effects lead to pathological changes of the RBCs such as resetting, clumping and micro-adhesion resulting in severe vital organ damage including the kidney, lungs and the brain (cerebral malaria) (Plewes, Turner, & Dondorp, 2018). Some key clinical manifestations include, prostration, impaired consciousness, respiratory distress, multiple convulsions, circulatory collapse, abnormal bleeding and haemoglobinuria(Perkins et al., 2011; WHO, 2019).

2.4.3 MALARIA ERADICATION APPROACHES

A comprehensive approach in the fight against malaria involves three main determinants; the mosquito, the environment and the plasmodium parasite.

2.4.3.1 Vector Control

The mosquito plays a very pivotal role in the life cycle of the malaria parasite, hence quite crucial to the survival and longevity of the parasite. There are two main methods of vector control recommended by the WHO; Indoor Residual Spraying (IRS) and the use of Long-Lasting Insecticide Treated Nets (LLITNs) and these account for about 60% of global investment into malaria eradication, thus highlighting their cruciality. Other interventions include the use of mosquito repellants and larvicidal agents with the former comprising both chemical and biological control methods (WHO, 2015). The use of chemical larvicidal agent which was the mainstay over the past decades is gradually losing grounds to the latter due to factors such as high environmental and ecological toxicity and the development of resistant mosquito strains (Rathy, Sajith, & Harilal, 2015).

2.4.3.1a Indoor Residual Spraying

The mosquito as a vector possesses either endophilic (prefers indoor environment) or exophilic (prefers outdoor environment) traits. IRS as a vector control method has been found to be highly effective against the endophilic mosquitoes and it involves the introduction of long-lasting insecticides on potential resting surfaces of mosquitoes such as ceilings, walls and other interior surfaces (GHS, 2018). Even though, IRS has been proven to be quite effective, there has been concerns of resistance by the WHO Global Malaria Program, thus key factors such as vector identification, feeding and resting behavior should be considered in the choice of a particular type to help reduce resistance (WHO, 2015).

2.4.3.1b Long Lasting Insecticide Treated Nets

The use of LLITNs occupies a crucial contribution gap especially for susceptible groups such as pregnant women and children under five years. It is designed to serve as a barrier for prevention of the bite of mosquitoes, hence reducing malaria transmission. Evidence shows that it is beneficial to the health of the mother throughout pregnancy and postpartum (WHO, 2015).

2.4.3.1c Chemical Control

In the past, the use of several chemicals have been employed in the mosquito control of malaria, including DDT, mercuric compounds, Paris Green (Copper Acetoarsenite) , phenols, petroleum oils, naphthalene, cresols, calcium arsenate and nicotine sulfate (Rodríguez-Pérez, Howard, & Reyes-Villanueva, 2012). Most of these chemicals have been found over the years to be extremely harmful to the environment. Key amongst them is DDT which has the ability to reside in humans for a long time leading to disorders such as breast, liver and pancreatic cancer, and various organ damages such as the kidney, nervous and reproductive system (Raghavendra, Barik, Reddy,

Sharma, & Dash, 2011). Apart from their detrimental effects on humans, these insecticides also destroyed aquatic habitats. Also studies have shown the emergence of resistant mosquito strains hence rendering most of these insecticides ineffective (Sokhna, Ndiath, Rogier, & Infection, 2013). Thus, these factors and many others have necessitated the advocacy for biological methods in the malaria vector control due to safety and benefits to the environment.

2.4.3.1d Biological Control

The introduction of fungi which have the ability to act as parasites to species of the *genera Coelomomyces, Culicinomyces, Beauvaria, Metarhizium, Lagenidium and Entomopthora* has been useful in the management of the mosquito (Scholte, Knols, Samson, & Takken, 2004). Through external contact with insect's cuticle, it inhibits its growth, although it has a slower killing rate than insecticides, studies have shown it to be effective in eradicating insecticide-resistant strain which is so crucial in suppressing these strains completely over a period of time (Thomas & Read, 2007).

Another biological approach is the use of bacterial agents such as *B. thuringiensis* and *B. sphaericus* which destroy the mosquito larvae and are also economically cheap, locally manufactured and easily handled (Charles & Nielsen-LeRoux, 2000).

Alternatively there can be the employment of the fish *Gambusia affinis* which feeds on the mosquito and larvae thus leading to depletion of their numbers over time (K. J. E. H. P. Walker, 2002).

Other methods include:

- the adoption of a fungus known as *Metarhizium pinghaense* which has been genetically modified to release a deadly venom similar to that produced by the funnel-web spider leading to drastic decline of the ecological anopheles numbers (BBC, 2019).
- The use of the shrub *Ocimum americanum* to drive away mosquitoes (Dembo, 2012).

2.4.3.2 Environmental Approach

The need for personal and environmental hygiene cannot be overemphasized in the fight against malaria. During the rainy season, factors such as high humidity (<60%) and optimum temperature (18-32°C) help mosquitoes to breed faster. Furthermore, rain water accumulated in stagnant places such as tires, bowls, construction sites, mining sites serve as ideal breeding grounds for the mosquito, thus the need to tidy up our environment to help break the chain of transmission (K. Walker, Lynch, & entomology, 2007).

2.4.3.3 The Chemotherapy Approach

Currently, classification of malaria chemotherapeutic agents in relation to their chemical structure include:

(i) The use of Aryl aminoalcohol-containg compounds such as: lumefantrine, amodiaquine, quinine, quinidine, chloroquine, mefloquine, halofantrine, lumefantrine, piperaquine, etc.

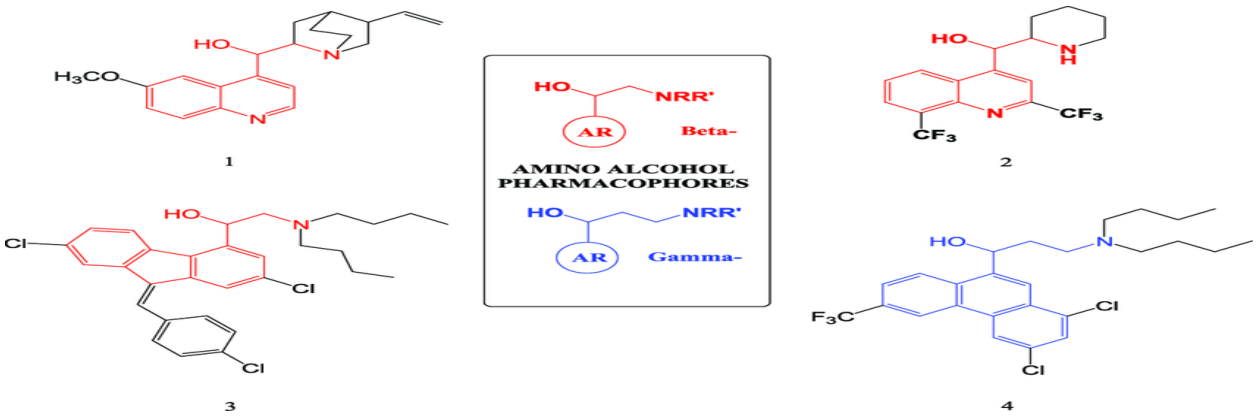


Figure 2.4: : Chemical Structure of Aryl Amino Antimalarial Agents, (1) Quinine, (2) Mefloquine, (3) Lumefantrine, (4) Halofantrine

(Retrieved from https://www.researchgate.net/figure/Antimalarial-drugs-with-amino-alcohol-moiety-as-a-pharmacophore-1-quinine-2_fig2_308860763)

(ii) The second groups are the antifolate compounds such as: pyrimethamine, proguanil, chlorproguanil, trimethoprim.

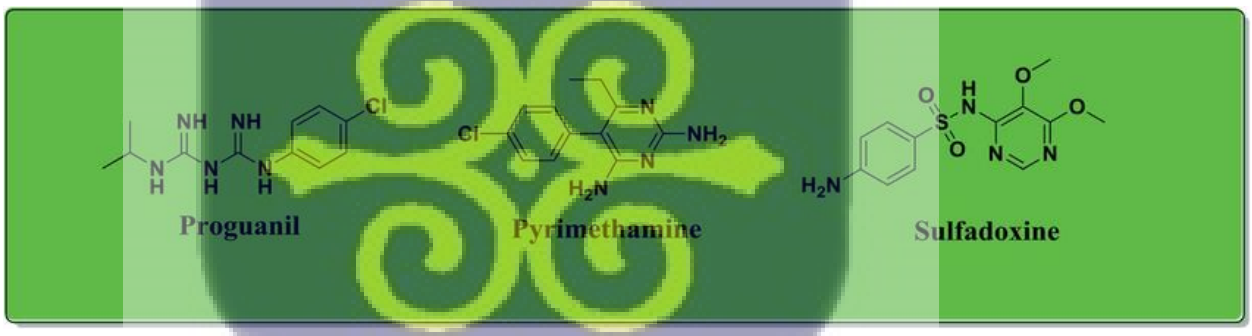


Figure 2.5: Chemical Structure of Antifolate Antimalarial Agents

(Retrieved from https://www.researchgate.net/figure/Chemical-structures-of-antimalarial-antifolates_fig2_303904807)

(iii) The last class are the artemisinin-based compounds which include: artemisinin, dihydroartemisinin, artemether and artesunate.

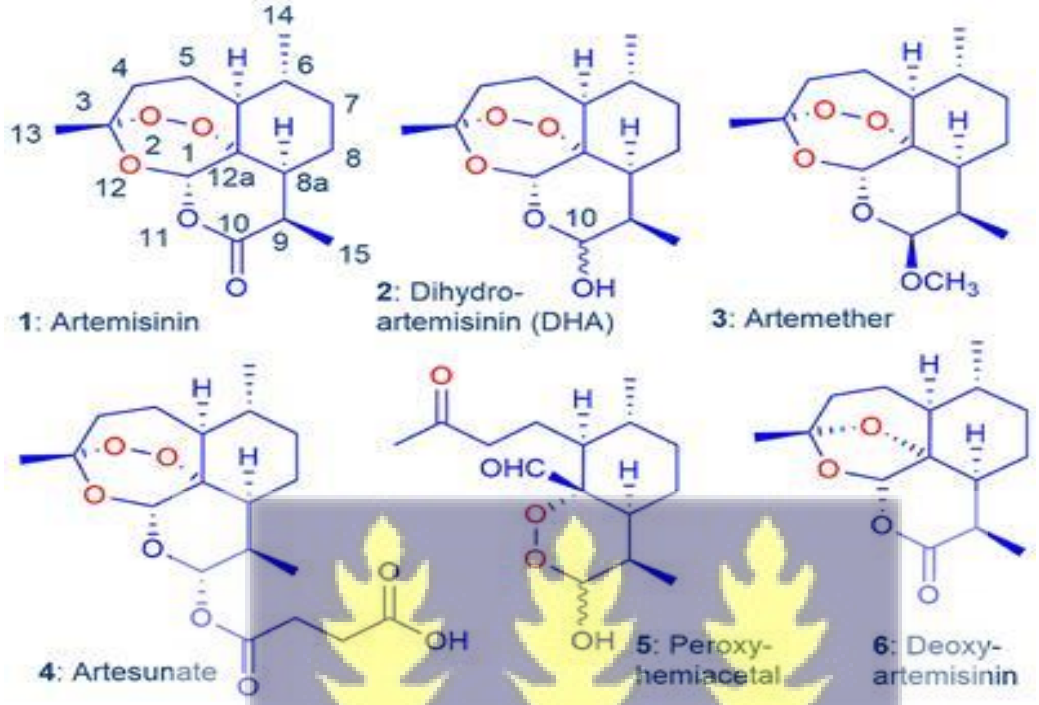


Figure 2.6: Chemical Structure of Artemisinin-based Antimalarial Agents

(Retrieved from https://www.frontiersin.org/articles/10.3389/fchem.2019.00901/full)

2.4.3.3a Chloroquine

In the past years, chloroquine was one the main antimalarial agents until several cases of resistance were reported. Its antimalarial activity faded over the years due to mutations of the *P. falciparum* Chloroquine Resistant Transporter (pfCRT), an integral protein present in the parasite's internal digestive vacuole causing the efflux and decreased accumulation of the drug in the food vacuole of the parasite (Martin et al., 2009; Summers et al., 2014).

However, there are other quinolone-containing antimalarials such as; quinidine and quinine which are still useful in the management of malaria (Fan et al., 2018; Winter et al., 2008).

Chloroquine which is a weak base accumulates in the food vacuole of the parasite via ion-trapping where it exerts its pharmacological action (Loria, Miller, Foley, & Tilley, 1999). Upon oral administration it is acted on by cytochrome p450 (CYP450) enzymes by the process of de-alkylation into two main products namely desethylchloroquine and bis desethylchloroquine with a prolonged elimination half-life between 20 to 60 days and an extensive distribution (200 – 800 L/kg) in to various parts of the body including the nails and eye (Karunajeewa et al., 2010; Yamada, Hidefumi, Shion, Oshikata, & Haramaki, 2011).

Apart from its antimalarial property, other areas which have found chloroquine to be quite resourceful in recent years is in the treatment of rheumatoid arthritis and systemic lupus erythematosus, especially its hydroxylated form (hydroxychloroquine). Currently it has also been recommended for the possible management of a disease caused by the variant corona virus strain (SARS-CoV2) (Gao, Tian, & Yang, 2020; Touret & de Lamballerie, 2020).

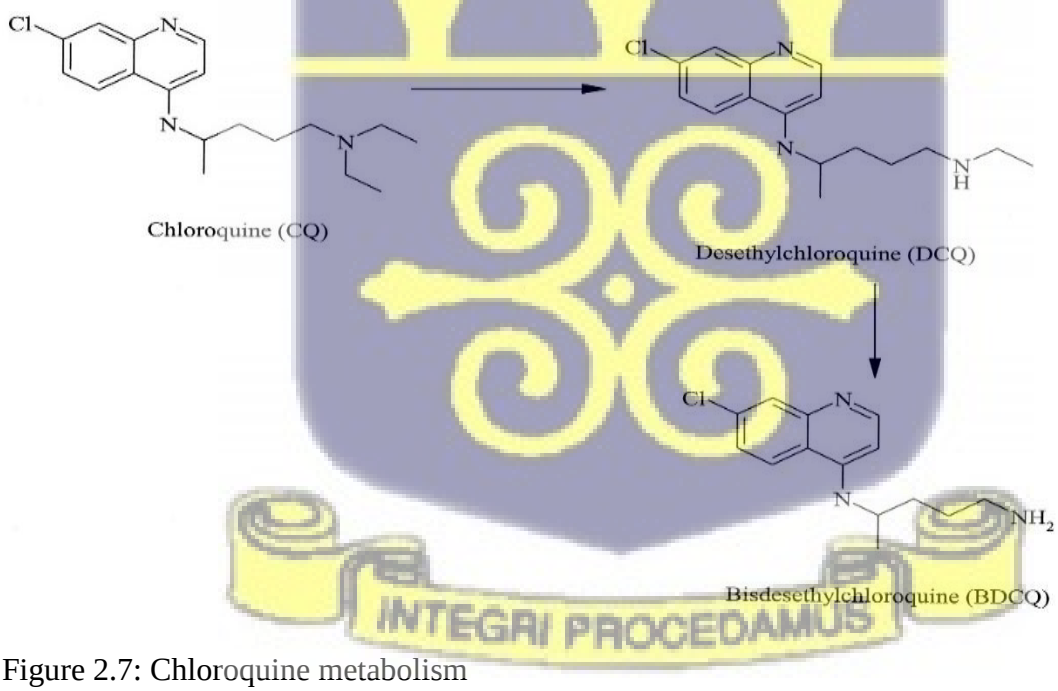


Figure 2.7: Chloroquine metabolism

(Retrieved from https://dmd.aspetjournals.org/content/31/6/748)

2.4.3.3b Quinine

Quinine is a naturally occurring alkaloid obtained from the bark of the cinchona tree and recognized as an approved drug by the WHO in curing malaria especially in the first trimester of pregnancy in uncomplicated malaria and in severe malaria due to *P. falciparum*. Quinine, just like chloroquine is a weak base and a schizonticide which acts by causing the accumulation of toxic haem in the food vacuoles of the malaria parasite hence causing the death of the parasite. When taken orally, 76- 88% absorption is achieved with a peak concentration occurring within 1-3 hours. It has high protein binding of about 70% and metabolized by the liver enzymes to 3-hydroxyquinine with an elimination half-life of approximately 18 hours (Pukrittayakamee et al., 2003). Despite the various side effects associated with this drug such as tinnitus, hypoglycemia, cardiac arrhythmias, eye and hearing impairment, it is one of the mainstays in the treatment of malaria (GHS, 2019).

2.4.3.3c Proguanil

Proguanil is an anti-plasmodial drug which is a synthetic biguanide pyrimidine derivative which is active against pre-erythrocytic hepatic forms of *P. falciparum*. It is generally well tolerated, it is a prodrug which is converted with the help of hepatic enzymes to active metabolite cycloguanil, an inhibitor of dihydrofolate reductase (DHFR) which is an integral component in the folic acid metabolism needed for growth and reproduction by the parasite (McGready et al., 2003). It has a peak concentration time of about 3 hours after oral administration and a plasma half-life of about 12-16 hours and its excreted in the urine and faeces in both unchanged and active form. Proguanil is used prophylactically in pregnant women and non-immune individuals at risk of exposure. It is also used in combination with atovaquone in malaria treatment (Edstein et al., 2005).

2.5 ARTEMISININ COMPOUNDS AND DERIVATIVES

The *Artemisia annua* plant belongs to the family *Asteraceae* with a brownish stem with average total plant height of 30-100 cm with a leaf length of approximately 3-5 cm. The artemisinin chemical compound is extracted from the stems, leaves and flowers of *Artemisia annua* commonly known as sweet wormwood or qinghaosu in China (Graham et al., 2010; Kumar et al., 2004). They are currently the most widely used group of compounds against malaria due to their high efficacy, tolerability and safety profile (WHO, 2019).

Figure 2.8: The *Artemisia annua* plant

(Retrieved from https://www.artennua.com/artemisia-annua/#)

The artemisinin compound is a sesquiterpene lactone, which acts on the blood schizonts of the plasmodium parasite and also on the gametocytes. It works by the release of free radical as a result of the breakdown of their endoperoxide bridge in their chemical structure. These free radicals released cause oxidative stress on the parasite thus leading to its death. Artemisinin and its derivatives are use in several pharmaceutical dosage forms such as tablets, powders for reconstitution into suspensions, injectables and suppositories (Chaturvedi, Goswami, Saikia, Barua, & Rao, 2010).

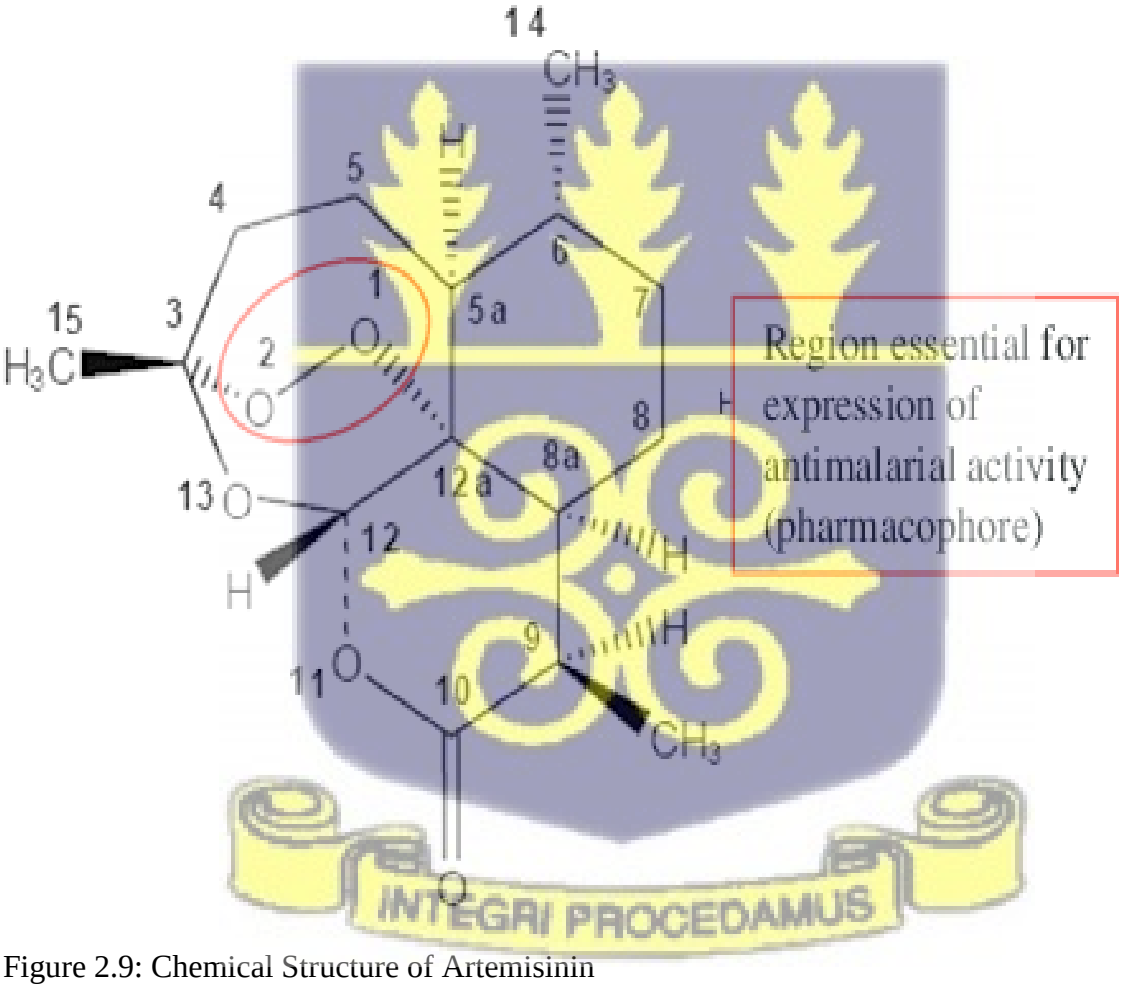


Figure 2.9: Chemical Structure of Artemisinin

(Retrieved from https://www.rainforest-alliance.org/species/cacao)

2.5.1 ARTEMISININ-BASED COMBINATION THERAPY (ACT)

In recent years ACTs have become the cornerstone in our fight against malaria. ACT refers to a group of antimalarial agents used as first line drugs in the management of uncomplicated malaria in various countries worldwide including Ghana. The WHO recommends five groups of ACTs in the treatment of uncomplicated *P. falciparum* malaria;

- Artemether/Lumefantrine
- Artesunate/Amodiaquine
- Dihydroartemisinin/Piperaquine
- Artesunate/Mefloquine
- Artesunate/ Sulfadoxine-Pyrimethamine

The artemisinin compounds in ACTs provide a faster onset of action for rapid parasite clearance but have a short elimination half-life followed by the longer acting partners to clear the remnant parasites and providing some form of post-treatment prophylaxis to reduce incidence of relapse (Grueninger & Hamed, 2013). A three-day treatment regimen is the recommended duration of all patients to help provide sufficient drug efficacy and minimize the risk of drug resistance (Mutabingwa, 2005).

In Ghana the approved drug combinations are Artemether-Lumefantrine (A/L), Artesunate-Amodiaquine (A/A) and Dihydroartemisinin-Piperaquine (DHAP) (GHS, 2018).

2.5.2 Pharmacology of Artemether/Lumefantrine

Artemether/Lumefantrine, just like the other ACTs is derived from the parent compound known as artemisinin. Research has established a therapeutic synergy between Artemether and Lumefantrine in the efficient plasmodium parasite clearance, hence the combination (Fan et al., 2018; Makanga & Krudsood, 2009; Pelgrift & Friedman, 2013).

In the body, artemether is metabolized into the active metabolite dihydroartemisinin, which both work against the erythrocytic stage of *P. falciparum* by inhibiting nucleic acid and protein synthesis and also causing oxidative stress on the parasite through the release of free radicals. Artemether has a rapid onset of action with peak concentration occurring approximately 2 hours after oral administration, hence provides rapid symptomatic relief by reducing the number of malaria parasites (Murray & Bennett, 2009; Sisowath et al., 2007). It is highly bound to plasma protein, 95.4% and 76% with half-lives of 1.6-2.2 hours and 1.6-2 hours respectively for the parent compound and metabolite (Lefèvre & Thomsen, 1999).

Lumefantrine has a much longer duration of action of about 3-4 days and is believed to clear residual parasites (Dodoo et al., 2009; Pelgrift & Friedman, 2013).

Lumefantrine is a blood schizonticide active against erythrocytic stages of *Plasmodium falciparum* by inhibiting the conversion of toxic haem to non-toxic haemozoin, thus the accumulation of the heam becomes toxic to the plasmodium parasite resulting in the death of the parasite. It is metabolized extensively by hepatic enzymes to the active metabolite desbutyl-lumefantrine. Lumefantrine has an elimination half-life of about 4.5 days with protein binding of about 95%. Some common side effects of A/L therapy include; anorexia, vomiting and cough and

adverse effects include QT prolongation, splenomegaly and hypersensitivity reactions. Both Artemether and Lumefantrine tend to exhibit higher parasite clearance when properly absorbed largely due to high plasma concentration resulting in good treatment outcome (Abay, Tilahun, Fikrie, & Habtewold, 2013; Abuaku, Duah, Quaye, Quashie, & Koram, 2012; Adeel et al., 2015).

2.5.2.1 Challenges of Artemether Lumefantrine

One of the main challenges of A/L is poor GI absorption. This is because it is highly dependent on dietary fat for GI absorption. Evidence indicates a two- and sixteen-fold increase in absorption of Artemether and Lumefantrine respectively in the presence of a fatty diet (Byakika-Kibwika et al., 2010; Djimdé & Lefèvre, 2009). However, due to loss of appetite, patients are not able to eat properly before taking the drug, hence leading to treatment failure as a result of poor absorption (Färnert et al., 2012). Additionally, there have been emerging reports of drug resistance associated with poor GI absorption which is linked with multidrug resistant gene *pfmdr1* 184F resulting in altered in vitro and in vivo response to arylaminoalcohols (Sisowath et al., 2007).

2.6 THE COCOA PLANT

The cocoa plant with a botanical name; *Theobroma cacao* is a tropical plant with a geographical distribution in the Amazons, Tropical Asia and West Africa.

Kingdom: Plantae

Class: Equiseptosida

Order: Malvaceae

Family: Sterculiaceae

Genus: Theobroma

PICTORIAL DESCRIPTION OF COCOA

Figure 2.10: Theobroma cacao *plant*

(Retrieved from https://www.rainforest-alliance.org/species/cacao)

2.6.5 Processing and Preparation Of UNCP

The harvested cocoa pods are firstly de-podded to obtain the beans, followed by fermentation for about 6 – 10 days. The fermented beans are then dried openly usually under the sun, then roasted which helps to enhance the flavour and colour of the cocoa beans. The beans are then winnowed to remove the shells in order to obtain the nibs. They are pressed to extract the butter followed by grinding and pulverization to a dry powder which is then packed.

Figure 2.11: Unsweetened Natural Cocoa Powder

(Retrieved from: https://www.dreamstime.com/ripe-cocoa-pod-nibs-beans-setup-background-image102220144)

2.6.7 Health Benefits of UNCP

Flavonoids present in cocoa have been discovered to possess numerous pharmacological activities including; anti-ageing, anti-bacterial, anti-fungal, anti-inflammatory, anti-diabetic, anti-hepatotoxic, anti-allergic, anti-thrombotic, anti-hypertensive, anti-tumor, CNS stimulant and myorelaxant, (Osakabe et al., 2001). They also have effect on key inflammatory enzyme systems such as: lipoxygenase (LOX), cyclooxygenase (COX) and protein kinase C, (Shohaib, Shafique, Dhanya, & Divakar, 2011). Their vasodilatory action is due to the inhibition of phosphodiesterase resulting in the increase in cAMP thus leading to vasodilation and relaxation of the bronchial muscles.

Major dietary sources of flavonoids include tea (green and black), red wine, apples, tomatoes, cherry, onions, thyme, parsley, soya beans, other legumes, grape fruits, oranges, lemons, ginkgo, dark chocolate and Neem (*Azadirachta indica*) (Sokpor et al., 2012). Flavonoids are also strong inhibitors of xanthine oxidase (XO), indicated in the treatment of atherosclerosis, gout, hyperuricemia and reperfusion injury (F. K. Addai, 2010). They have also been shown to inhibit the growth of various cancer cell lines like prostate cancer *in vitro* and reduce tumor development. The aldo-reductase inhibitive property of flavonoids is useful in diabetes-induced retinopathy and cataract. Catechins specifically act as anti-ulceric agents by inhibiting the H^+/K ATPase (Shohaib et al., 2011).

Additional benefits include anti-asthmatic activity as a result of the presence of theobromine and theophylline which are xanthine derivatives needed for dilation of the pulmonary airways (Awortwe, Asiedu-Gyekye, Nkansah, & Adjei, 2014). There is also the possibility of UNCP in the inhibition of key immune response implicated in asthma –(IgE) and reduction of cytokines such as TNF-α and IL-10. Records of significant decrease in the these immune markers have been observed in the cocoa-treated than the untreated allergy-induced rats thus suggesting the significant role of flavonoids present in UNCP in the management of allergies and also found to possess immunostimulatory effect due to the presence of key micro elements such as copper, zinc and manganese (Abril-Gil et al., 2012; Camps-Bossacoma et al., 2017).

2.6.8 Antimalarial Property of UNCP and Possible Synergy with AL

Anecdotal reports indicate that a regular daily intake of UNCP reduces episodic malaria occurrence and this has been demonstrated *in vitro* and *in vivo* antimalarial experimentations (F. K. Addai, 2010; Amponsah et al., 2012; Ishaq & Jafri, 2017). This antimalarial activity has been linked with the rich presence of flavonoids and alkaloids.

Furthermore, it is also abundant in fatty acids such as palmitic acid, stearic acid and oleic acid (Ortega et al., 2009), which could aid the absorption of AL.

2.8 QUALITATIVE AND QUANTITATIVE ANALYTICAL METHODS

2.8.1 Phytochemical Screening

Phytochemicals are primary and secondary metabolites found in various parts of plants and have been associated with medicinal properties of most plants. Some of these include tannins, glycosides, flavonoids, phenols and reducing sugars.

2.8.1.1Flavonoids

They are a group of polyphenols consisting of a variety of chemical structures and properties abundant in foods such as citrus fruits, berries, apples, legumes and cocoa. Several studies have shown the various health benefits of flavonoids such as anti-inflammatory, anticancer properties, anti-oxidant property due to their free radical scavenging capacity (Ballard & Junior, 2019; Patel, 2008; Yao et al., 2004) .

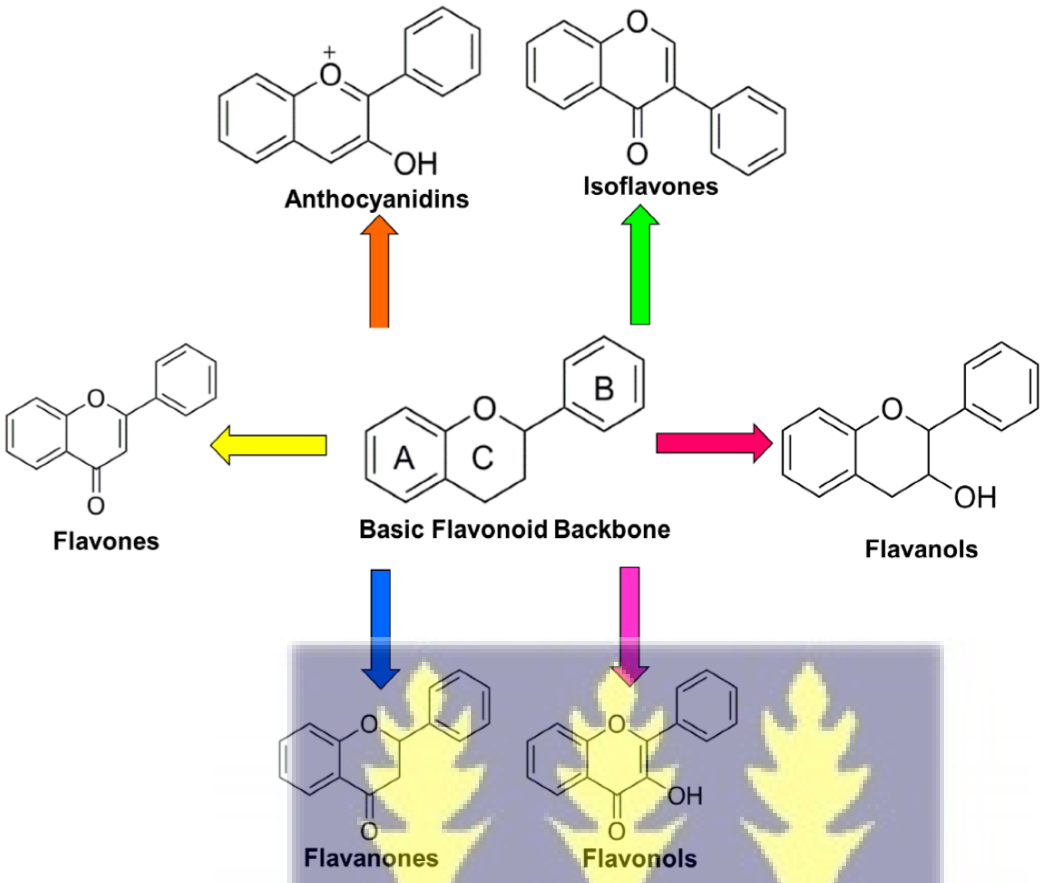


Figure 2.12: Basic Structures of Flavonoid Subclass

(Retrieved from https://www.mdpi.com/2076-3921/7/12/187/htm)

2.8.2 Total Fatty Content Assay

Fats, just like other essential nutrients such as proteins and carbohydrates play a crucial role in the nutritional benefits of any food product. Additionally, knowledge about its influence on drug-food interaction has been quite beneficial over the past few years in the field of pharmacology especially in aiding absorption of fat soluble drugs (Custodio, Wu, & Benet, 2008).

There are several methods of determining the fat content of food products with the common approaches including: solvent extraction, gravimetric method and gas chromatography. These methods have both their advantages and shortfalls, in terms of accuracy, the gas chromatography method is the ideal choice however it is also costly and needs a lot of expertise. Conversely, the solvent extraction is quicker and less costly (Lehotay & Hajšlová, 2002; Tranchida et al., 2013). The solvent extraction method employs the use of either acid or base hydrolysis or both in the breakdown and the release of fatty acids for quantification. The Soxhlet apparatus is commonly used in this method of fat analysis.

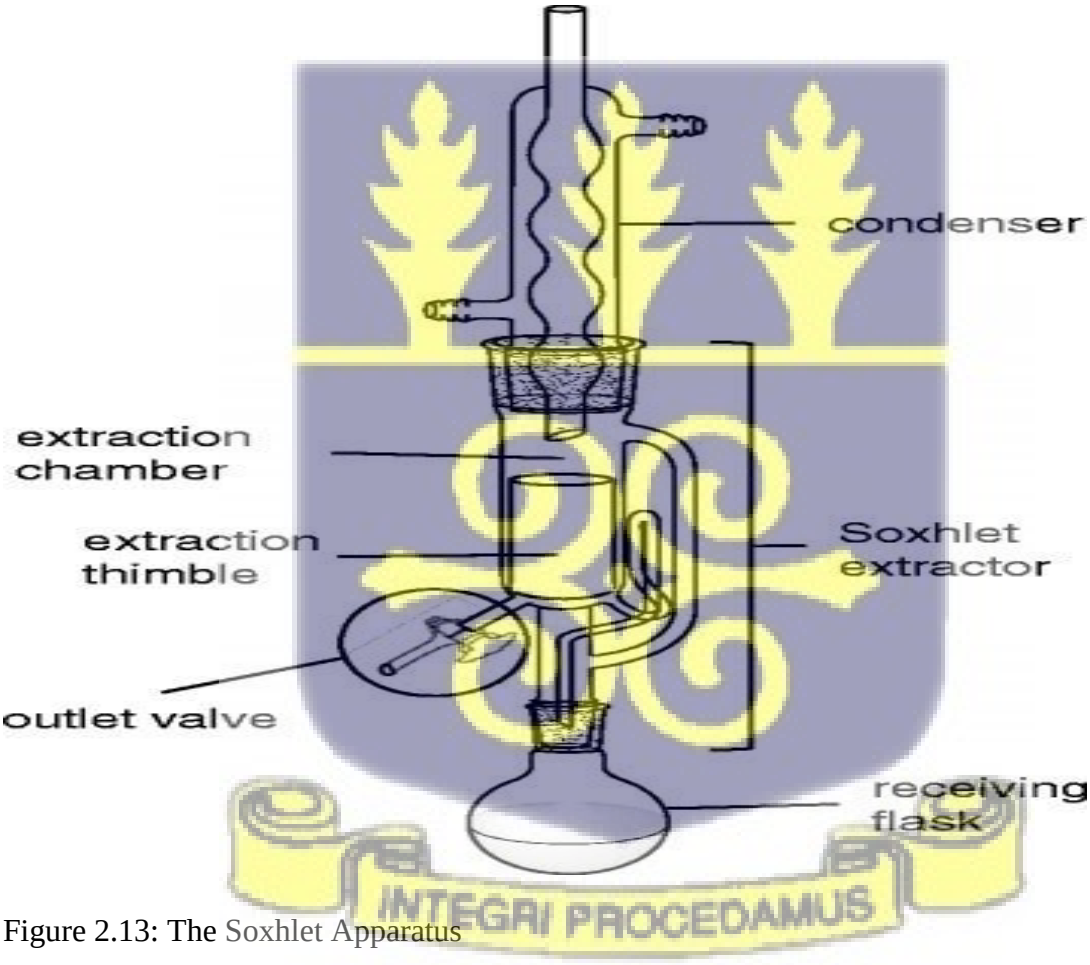


Figure 2.13: The Soxhlet Apparatus

(Retrieved from https://indiafeelsblog.wordpress.com/2015/09/01/-soxhlet-extraction-method/)

2.8.3 HPLC Method of Drug Quantification

Since the early 90's, chromatography has become the bedrock of analytical chemistry in the separation, detection and quantification of biochemical constituents. The concept of chromatography has only evolved but has never changed It involves the separation of individual constituents present in a mixture based on their degree of affinity (adsorption) for the stationary and mobile phase respectively. HPLC, which is only a modernized version of this concept has been the breakthrough in the field of drug discovery, biochemistry and forensics has contributed to the advancement of science over the past few years (Gerber et al., 2004; Yandamuri et al., 2013).

Components of the HPLC Apparatus

The HPLC machine is comprised of key components that perform specific role in achieving the desired results, these parts work in a well-coordinated manner. Below are the various components and their functions.

- The Pump: It is responsible for the propelling of the mobile phase or HPLC solvent onto the stationary phase at a constant flow rate as specified by the method of analysis.
- Injector: It plays the role of introducing the sample under examination into the flow stream of the mobile phase.
- HPLC Column: It serves as the stationary phase and is mostly composed of silica which makes it either more polar or less polar than the mobile phase
- Detector: This is where the constituent eluted from the column is identified mostly composed of UV-Vis spectrometry, Flourescence, Photo-diode array or Mass Spectrometry.

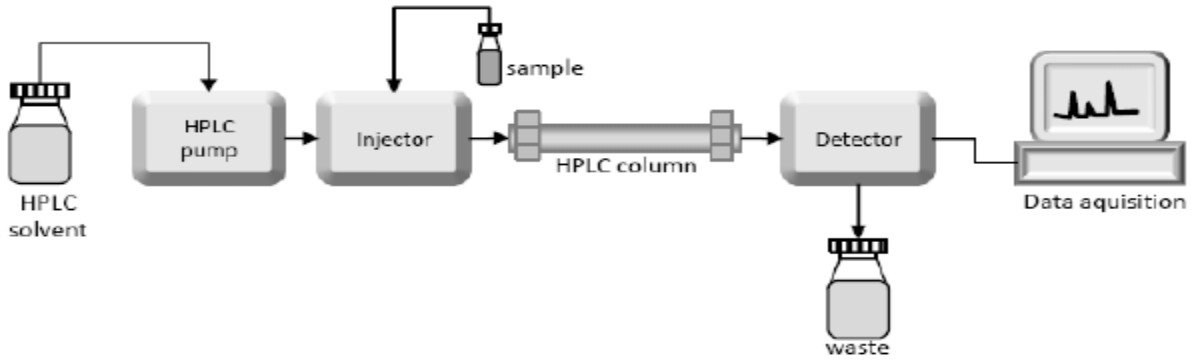


Figure 2.14: A Schematic Diagram of the Components of HPLC System

(Retrieved from: https://www.researchgate.net/figure/Schematic-diagram-of-the-High-Performance-Liquid-Chromatography-HPLC-system_fig2_236146377)

Some of the common types of HPLC include;

- Normal-phase chromatography: This is made up of a polar stationary phase bare silica with several -OH bonds and a less polar mobile phase, eg. Chloroform (Natsume et al., 2000).
- Reversed-phase Chromatography: Column is composed of a non-polar stationary phase of C-8 or C-18 carbon-chained silica polymer with a polar mobile phase. This concept is an opposite approach of the Normal-phase chromatography (Bartolomeo & Maisano, 2006; Kalyankar & Kakde, 2011).
- Size Exclusion Chromatography: Separates constituents on the basis of their molecular size. It is mostly used in the determination of tertiary and quaternary structure of proteins (Bajaj, Sharma, & Kalonia, 2004; Li et al., 2007).
- Ion- Exchange Chromatography: It is based on the affinity of ion constituents present in the sample of examination for charged sites of the stationary phase (Knudsen, Fahrner, Xu, Norling, & Blank, 2001; Rea, Moreno, Lou, Farnan, & analysis, 2011).

Liver Function Test

The liver is a vital organ in drug metabolism especially when administered orally, thus it is always imperative to examine the possibility of damage in experimentation involving drug products. Some of the common liver function test (LFT) includes; Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Aspartate Amino Transferase (AST), Albumin ALB), Gamma Glutamyl Transpeptidase (GGT) and Total Bilirubin. Elevation of these biomarkers beyond the acceptable range indicate the possibility of liver damage, although other tests may be needed to draw solid conclusions. This is because these biomarkers may be found in organs and tissues other the liver hence results may be misleading, nevertheless it is a good substitute to draw inference.

Table 2.1: Liver Biomarkers and their Sources

LIVER BIOMARKER	ORGAN/TISSUE SOURCES
AST	Liver, Heart, Skeletal muscles, Kidney, Brain, Red Blood Cells
ALT	Liver
ALP	Liver, Bone, Intestine, Placenta
BILIRUBIN	Liver
ALBUMIN	Liver

A/L has been implicated in liver damage by several toxicity studies manifesting histopathologically such as decrease in hepatic enzymatic antioxidant status (superoxide dismutase and catalase), as well as elevation of liver biochemical enzymes (Owumi, Gbadegesin, Odunola, Adegoke, & Uwaifo, 2015). A current in-house study performed by the Pharmacology Department, University of Ghana to ascertain the key culprit of hepatic damage indicate lumefantrine to be the main culprit at high doses.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 MATERIALS, CHEMICALS AND REAGENTS

The underlisted materials, chemicals and reagents were used in this study: Unsweetened Natural Cocoa Powder (Hords Brown Gold Natural Cocoa Powder®, Ghana), Artemether/Lumefantrine (Coartem® from Novartis Pharma, Switzerland), Artemether and Lumefantrine (Anhydrous API, purchased from IPCA Pharmaceuticals, India), Giemsa Stain, NaCl (Anhydrous salt, Analytical grade), Methanol (HPLC grade), Acetonitrile (HPLC grade), Ammonium Acetate, 0.2% Glacial Acetic Acid, Drug-free Human Plasma, 0.45 µm syringe filter (NSTR pk100).

3.2 PHYTOCHEMICAL SCREENING OF UNCP

A variety of phytochemical screenings were performed on the UNCP sample to help in the identification of the phytochemicals present. These tests include;

3.2.1 Test for alkaloids

About 0.2g of UNCP was extracted with ammoniacal alcohol. The extract was then filtered and evaporated to dryness. The residue was extracted with 1% aqueous sulphuric acid. The extract was filtered and rendered distinctly alkaline with dilute aqueous ammonia solution. The filtrate was shaken with chloroform and the chloroform extract separated. The chloroform was evaporated off the extract. The residue was dissolved in 1% sulphuric acid.

a) One drop of Mayer's reagent was added to the 1% sulphuric acid extract from above ad observed for the appearance of a white buff precipitate.
b) One drop of Dragendoff's reagent was added to the sulphuric acid extract from above and for the appearance of orange-red precipitate.

3.2.2 Test for saponins

The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells is used as a screening test for these compounds. Small quantity of UNCP sample was taken and shaken with water in a test tube. Frothing which persists on warming is taken as a preliminary evidence for the presence of saponins.

3.2.3 Test for Glycosides

An amount of 0.2 g of UNCP sample was weighed and extracted by warming with 5ml dilute sulphuric acid on a water bath for 2 minutes. The extract was filtered and the filtrate made distinctly alkaline with a few drops of 20% NaOH. The alkalinity was tested with a filter paper. 1 ml of Fehling's solution A and B was added to the filtrate and heated on a water bath for 2 minutes.

3.2.4 Test for Tannins

An amount of 0.2 g of UNCP was extracted with 25 ml of water for 5 minutes on the water bath. The extract was cooled, filtered and adjusted by volume to 25 ml.

a) To 1 ml of the extract, add 10 ml of water and 2-10 drops of lead acetate solution. The colour of the precipitate formed was noted.
b) To 1 ml of the extract, add 10 ml of water and 2-10 drops of 1% ferric chloride solution. The colour of the precipitate formed was noted. A blue-black, green or blue-green coloration is taken as evidence for the presence of tannins.
c) To 3 ml of the diluted solution, 3ml of 1% gelatine solution was added.

3.2.5 Test for Flavonoids

About 0.2 g of UNCP sample was weighed in separate test tubes and dissolved with diluted Sodium hydroxide and diluted Hydrochloride and observed for yellow solutions that turn colourless. This indicates the presence of flavonoids

3.2.7 Test for Reducing Sugars

Two millilitres of aqueous extract and 5 ml of distilled water were stirred and filtered together. The filtrate was boiled with 3-4 drops of Fehling's solution A and B for 2 minutes and observed for orange red precipitate which indicates the presence of reducing sugars.

3.2.8 Test for Phenol

About 0.2 g of UNCP sample was weighed and treated with 5% ferric chloride and observed for the formation of deep blue colour which indicates the presence of phenol (Trease & Evans, 1989).

3.3 TOTAL FAT CONTENT ASSAY

Two grams (W1) of UNCP was weighed into an extraction thimble subsequently plugged with fat-free cotton wool. The thimble was placed in the extraction tube allowed to slide down side of the tube. A dried Soxhlet flask with fat-free boiling chips was weighed (W2) and filled with petroleum ether (B.P 40-60°C) to almost half of its volume- this was performed in the fume chamber. The extractor was assembled on the rack, the heater and condenser water turned on. The setup was allowed to reflux rapidly for about 3 hours, the extractor was then disconnected, with the thimble lifted up to the top of the tube with tongs and clips to the side for draining.

The thimble was then removed, apparatus reconnected and distillation continued using the siphon to reclaim the ether. The flask was removed and transferred to a steam bath for evaporation of the final few millilitres of ether followed by drying the flask in an oven at 60°C overnight. The flask was then cooled in a desiccator and weighed (W3). The difference in weight (W3 − W2) corresponded to the fat content of the sample and the percentage fat was calculated using the formula below:

$$Percentage\ fat = \frac{W3 - W2}{W1}\ X\ 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(3)$$

3.4 STUDY DESIGN

This was an experimental study consisting of two phases. Phase one was designed to determine the antimalarial effect of A/L with varying doses of UNCP in *P. berghei*-infected animals, whiles phase two involved the measurement of the plasma concentration of A/L when co-administered with UNCP. It must however be noted that different sets of the rats were used for each phase.

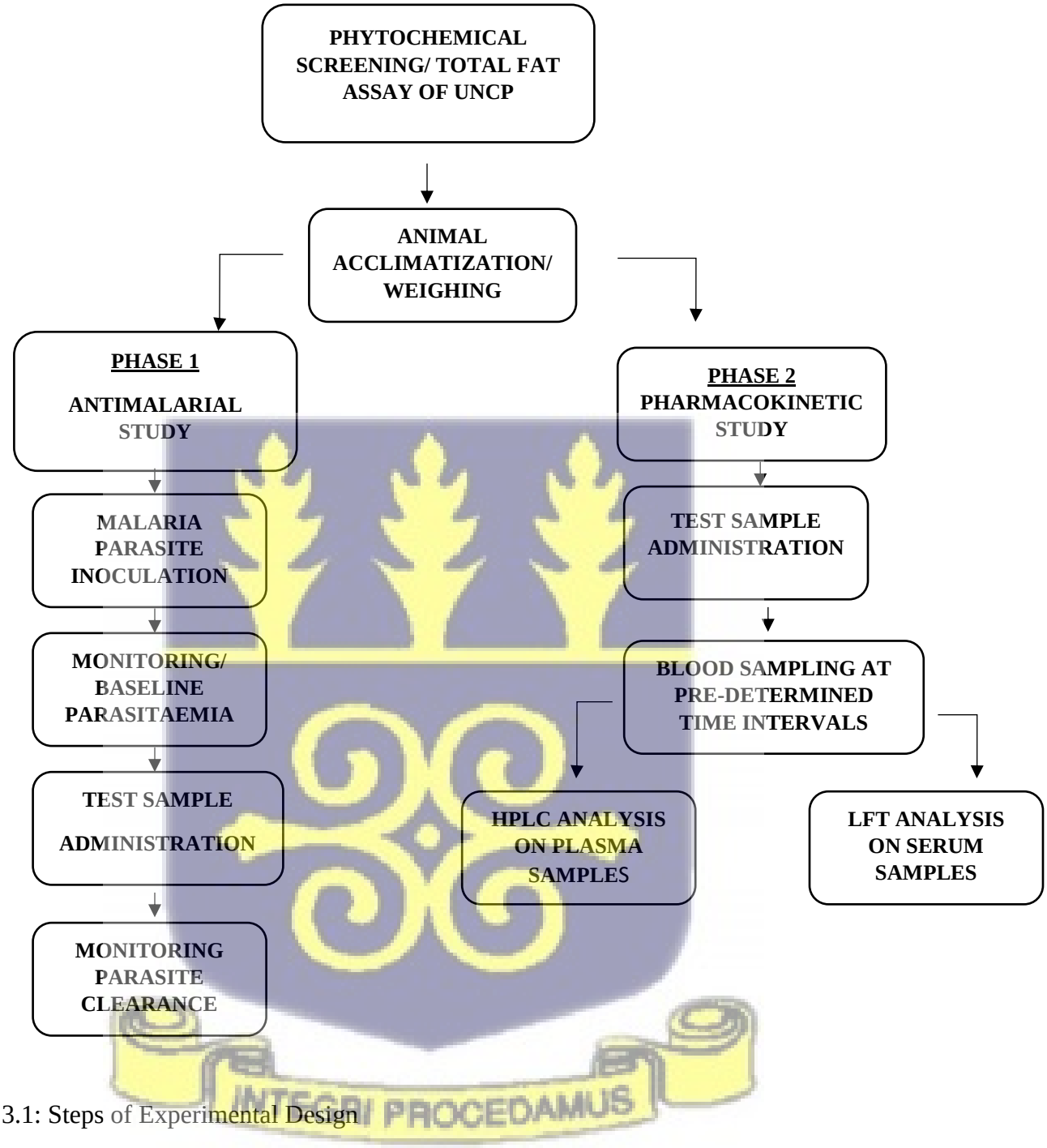


Figure 3.1: Steps of Experimental Design

3.5 ANIMAL EXPERIMENTATION AND HOUSING

The study subjects used were 8 weeks old male Spague-Dawley (SD) rats with an average weight of 250 g – 300 g obtained from the Department of Animal Experimentation of Centre for Plant Medicine Research, Mampong after ethical clearance approval was received from the Ethical and Protocol Review Committee (EPRC), College of Health Sciences – Korle-Bu with protocol number of *CHS-Et/M5-5.3/2019-2020* (Refer to Appendix 3).

The animals were kept in clean stainless-steel cages at the Animal House of the Department of Medical Microbiology, University of Ghana, Korle-Bu in enclosed, well-ventilated rooms with proper lighting under hygienic conditions. Each rat occupied a minimum space of 2 cubic feet (61 cm x 31 cm x 31 cm) with soft wood shavings as bedding for their comfort. They were fed with normal pellet diet (AGRIMAT, Kumasi), given water ad libitum, and maintained under standard laboratory conditions (temperature 25 ± 1°C, relative humidity 60-70%, and 12-hour light-dark cycle). All feeding and water troughs were regularly cleaned to prevent contamination. The animals were acclimatized for a week under the above conditions before experimentation. All animal procedures and techniques used in this study were in accordance with the National Institute of Health guidelines for the Care and Use of Laboratory Animals (N.R.C., 2010).

3.6 GROUP SAMPLING

The assignment of animals into each group was by simple random sampling. The total number of groups and the group size chosen helped capture the full scope of the research and was justified by the statistical analysis employed at a level of acceptable error.

3.7 ANTIMALARIAL STUDIES (PHASE ONE)

The test rats were randomly selected into 12 groups of 5 with each animal infected with 0.2 ml of parasitized erythrocytes containing 1×10^7 standardized A/L sensitive *P. berghei* obtained from

the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon. The parasite inoculation was performed via intraperitoneal injection. Animals were monitored until a mean baseline percentage parasitaemia of about 30% was achieved before test samples were administered to the various groups.

3.7.1 Parasite Monitoring

Monitoring of blood parasite levels was performed daily post-infection until the above baseline percentage parasitaemia was achieved on day 3 before test sample administration commenced. The choice of the above baseline was in accordance with other experimental works from literature. Parasite quantification was determined with the help of microscopy using the thick and thin film methods (Keita Alassane et al., 2017).

3.7.2 Test Sample Administration

The animals were weighed to determine the appropriate dose to administer, groups 1-5 were co-administered AL+UNCP, groups 6-10 given varying doses of only UNCP while group 11 and 12 constituted the negative and positive controls respectively. The test samples were administered orally per 2ml via oral gavage in fasted animals using distilled water as vehicle. A two-hour fasting pre- and post-sample administration was maintained to ensure that the presence of food does not affect the absorption of the test samples (Borrmann et al., 2010). Below are the various groups and their respective agents administered;

Table 3.1: Dose of test samples for respective groups

Groups	Artemether (mg/kg)	Lumefantrine (mg/kg)	UNCP (mg/kg)
1	2	12	300
2	2	12	600
3	2	12	900
4	2	12	1,200
5	2	12	1,500
6	-	-	300
7	-	-	600
8	-	-	900
9	-	-	1,200
10	-	-	1,500
11 DISTILLED WATER (2 ml)	-	-	-
12 COARTEM	2	12	-

3.73 Test Sample Dose Regimen

A twice daily dosing regimen for three days was employed as per the recommended protocol based on the following time intervals 0, 8, 24, 36, 48, 60 hours (WHO, 2019).

3.7.4 Blood Sample Collection

Prior to test sample administration, all the animals of the various groups were infected with *P. berghei* (NK65). This was performed by initial inoculation of donor rats with 0.2 mL of 10^7 parasitized red blood cells (RBCs) per µL intraperitoneally. A series of passages was performed until infection was fully established. Parasite monitoring was performed by obtaining blood from the tail vein of the infected animals, placed on a clean glass slide (All Pro Processed Microscope Slide, Cat # 7105, Surgifriend Medicals, Middlesex, England) and taken through the procedure for thick and thin film preparation for examination under a light microscope (Leica, Galen III, Cat# 317505; Leica Microsystems, Wetzlar, Germany) with immersion oil and ×100 objective lens (Mathison & Pritt, 2017). After the target parasite density was achieved, blood was drawn from the stock animals by cardiac puncture after anaesthesia and collected in heparinized tubes. It was immediately diluted using normal saline (0.9% NaCl) to obtain a standard inoculum. A volume of 0.2 mL of blood equivalent to 10^7 parasitized *P. berghei*-infected erythrocytes was introduced to all the experimental animals via intraperitoneal injection with parasite density monitored in terms of percentage parasitaemia for 3 days where the target parasite range (29% – 32%) was obtained before administration of test samples commenced (Cox, 1964; Nureye, Assefa, Nedi, Engidawork, & medicine, 2018). These values served as the baseline percentage parasitaemia for each group pre-sample administration. The thick film helped in the determination of parasite density calculated as follows:

$$\text{Parasite Density } (\mu\text{L}) = \frac{\text{Number of parasites}}{\text{200 White blood cells}} X\ 800 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(4)$$

3.7.5 Thin Blood Film

The collected blood drops on the glass slides were spread with the help of a clean spreader. This was done by allowing the blood to run along the edge of the spreader. The spreader was then firmly pushed along the slide at an angle of 45° with even contact on the surface of the other slide while the blood was being spread. The blood was allowed to dry for about 2 minutes and dipped in methanol for about 3-5 seconds to fix the cells on the slide. After drying, the smear was stained with 10% Giemsa stain for about 10-15 minutes, washed off with clean distilled water, allowed to dry and examined under a light microscope.

3.7.6 Microscopy

The slide was placed on a stage sitting the 100x objective lens and a drop of immersion oil applied at the middle of the thin film. The mechanical stage was racked up until the objective lens touches the immersion oil. The blood film was then examined along the edge of the film and then moving the slide inwards for one field up to 10 fields. The percentage parasitaemia was determined using equation (2) above (Manser, Olufsen, Andrews, & Chiodini, 2013).

3.8 PHARMACOKINETIC STUDY (PHASE TWO)

3.8.1 Animal Grouping

Thirty-five 8 weeks old male SD rats with an average weight of 250 g – 300 g were randomized into 7 groups (n = 5); five test groups, one positive and negative control group. Groups 1-5 were administered A/L in conjunction with varying doses of UNCP orally; 300, 600, 900, 1200, 1500 mg/kg respectively. Groups 6 and 7 were given Coartem® and distilled water, and represent the positive and negative control groups respectively. This was performed in healthy non-malarious animals.

3.8.2 Test Sample Administration

A/L dosing was done per body weight of animal. All test sample administration was done orally using oral gavage. A twice daily dosing (12 hourly) of A/L was administered with varying doses of UNCP for two days after which blood sampling began after the last dose administration at predetermined time intervals.

3.8.3 Blood Sample Collection

The first blood sample was drawn 30 minutes after the last dose administration, followed by the other time points. This was done by taking whole blood samples of volume about 500 µl from the tail vein of the rats at times of 0.5, 1, 2, 4, 8, 12, 24 hours into heparinized tubes over a 24-hour period. The samples were kept on ice packs immediately and centrifuged at 2,500 rpm for 15 minutes within one hour of collection. The supernatant which formed the plasma was collected and stored in labelled cryotubes at -20°C for HPLC analysis. On the last time point of blood collection, two sets of blood samples were collected; one for the routine drug plasma concentration analysis as described and the serum sample for liver function test (LFT) analysis respectively.

3.8.4 HLPC Analysis

3.8.4.1 Sample Preparation/Drug Extraction from Plasma

The stored plasma sample was thawed at room temperature for about 1-2 hours. Samples were vortexed in 15 ml screw capped polypropylene centrifuge tubes for 30 second. An amount of 1.5 ml of acetonitrile was added to precipitate plasma proteins, the mixture was further vortexed for 1 minute, and then centrifuged for 5 minutes at 10,000 × g. Supernatant was transferred into clean tubes and evaporated to dryness at 37 °C under a steady stream of nitrogen. The residue was reconstituted with 500 µl mobile phase and 20 µl injected into the column.

3.8.4.2 Instrumentation

HPLC analysis was performed on a LC-20AT (Shimadzu, Japan) liquid chromatograph equipped with an SPDM-20A UV-detector (Shimadzu, Japan). The separation was performed on a YMC-Pack Pro C18, 5 µm, 12 nm. A UV-Visible spectrophotometer model-1700 (Shimadzu, Japan) was used to determine UV-spectra of both compounds.

3.8.4.3 Chromatographic conditions

Column: YMC-Pack C18, 5 µm, 12nm. Length 150 mm, internal diameter 3.0 mm.

Mobile Phase A: Ion pair reagent A + Acetonitrile + TFA 490 + 510 + 1 (v/v/v). A volume of 1.0 ml of TFA was pipetted into a container containing 490 ml of ion pair reagent A and 510 ml of acetonitrile mixed well.

Mobile Phase B: Acetonitrile + TFA 1000 + 1 (v/v). A volume of 1.0 ml of TFA was pipetted into a container containing 1000 ml of acetonitrile and mixed well.

Needle Wash: Water + Acetonitrile + TFA 500 + 500 + 0.5 (v/v/v)

Table 3.2 : Gradient Program of HPLC Analysis

Time (min.)	Phase A (%)	Phase B (%)	Curve
0.0	100	0	-
12.0	100	0	Linear
16.0	48	52	Linear
20.0	48	52	Linear
20.1	100	0	Linear
25.0	100	0	Linear

Flow rate/Pressure: 1.0 ml/minute

Detection: UV, 210 nm for Artemether and 380 nm for Lumefantrine. UV response was collected at each response specified wavelength using individual detection channel or set the detector at 210 nm for the first 13minutes, then switched to 380 nm.

Sampling rate: Not less than 2 pts/sec

Column Temperature: 30ºC

Auto Sampler Temperature: 5ºC

Injection volume: 20 µl in each case, equivalent to about 4 µg of Artemether and about 24 µg of Lumefantrine in the reference solution.

Run time: 25 minutes

Ion Pair Reagent A: An amount of 3.77 g (20 mmol) of 1-Hexanesulfonic acid sodium salt in 1000 ml of water and mixed well. This solution was filtered through 0.45 µm membrane filter.

Extraction Solvent: Ion pair reagent A + De-ionized water + Acetonitrile + TFA 250 + 250 + 500 + 1.5 (v/v/v/v). A volume of 3.0 ml of TFA was pipetted in a container containing 500 ml of water and 1000 ml of acetonitrile mixed well.

Diluent: Ion Pair Reagent A + De-ionized water + Acetonitrile + TFA 250 + 250 +500 + 1.5 (v/v/v/v). A volume of 1.0 ml of TFA in a container containing 500 ml of ion pair reagent A, 500 ml of water and 1000 ml of acetonitrile mixed well.

3.9 LIVER FUNCTION TEST

A whole blood volume of about 1,000 µl was collected in paediatric clot activator gel tubes, allowed about a minute for blood to clot followed by the collection of about 500 µl of serum into labelled cryotubes for LFT analysis for levels of specific biochemical markers- Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Aspartate Amino Transferase (AST) and Albumin.

CHAPTER 4

4.0 RESULTS

4.1 PHYTOCHEMICAL SCREENING OF UNCP

Preliminary phytochemical screening revealed the presence of alkaloids, saponins, glycosides, tannins, flavonoids and phenols (Table 4.1).

Table 4.1: Phytochemical constituents of UNCP

PHYTOCHEMICAL TEST	OBSERVATION
Alkaloids	+
Saponins	+
Glycosides	+
Tannins	+
Flavonoids	+
Reducing Sugars	-
Phenol	+
Terpenoids	-

Key: Present (+), Not Detected (-)

4.2 TOTAL FAT ASSAY OF UNCP

Total fat analysis of UNCP showed a percentage fat content of 28.11% as shown in Table 4.2.

Table 4.2: Results of Total Fat Content of UNCP

TEST CONDUCTED	UNIT	RESULT
Total Fat Content	/100 g	28.11 g

4.3 UNCP ONLY GROUPS COMPARISON

The UNCP only groups showed a general increase in parasitaemia throughout the entire time frame with a steeper rise amongst the lower UNCP only doses than the higher doses (1,200 and 1,500 mg/kg) as shown in Fig. 4.1. However, the initial general increase in parasitaemia within 24 hours of sample administration across all the groups did not show any significant difference at times 0 and 8 hr as shown in Fig. 4.2 (a) and (b) respectively. But there was a significant difference for the higher doses of the UNCP i.e. 1,500 mg/kg ($F_{6,28}$=459, P <0.0001) and 1,200 mg/kg $F_{6,28}$=459, P < 0.0029) compared to the negative control group at 24 hours as shown in Fig.4.2 (c).

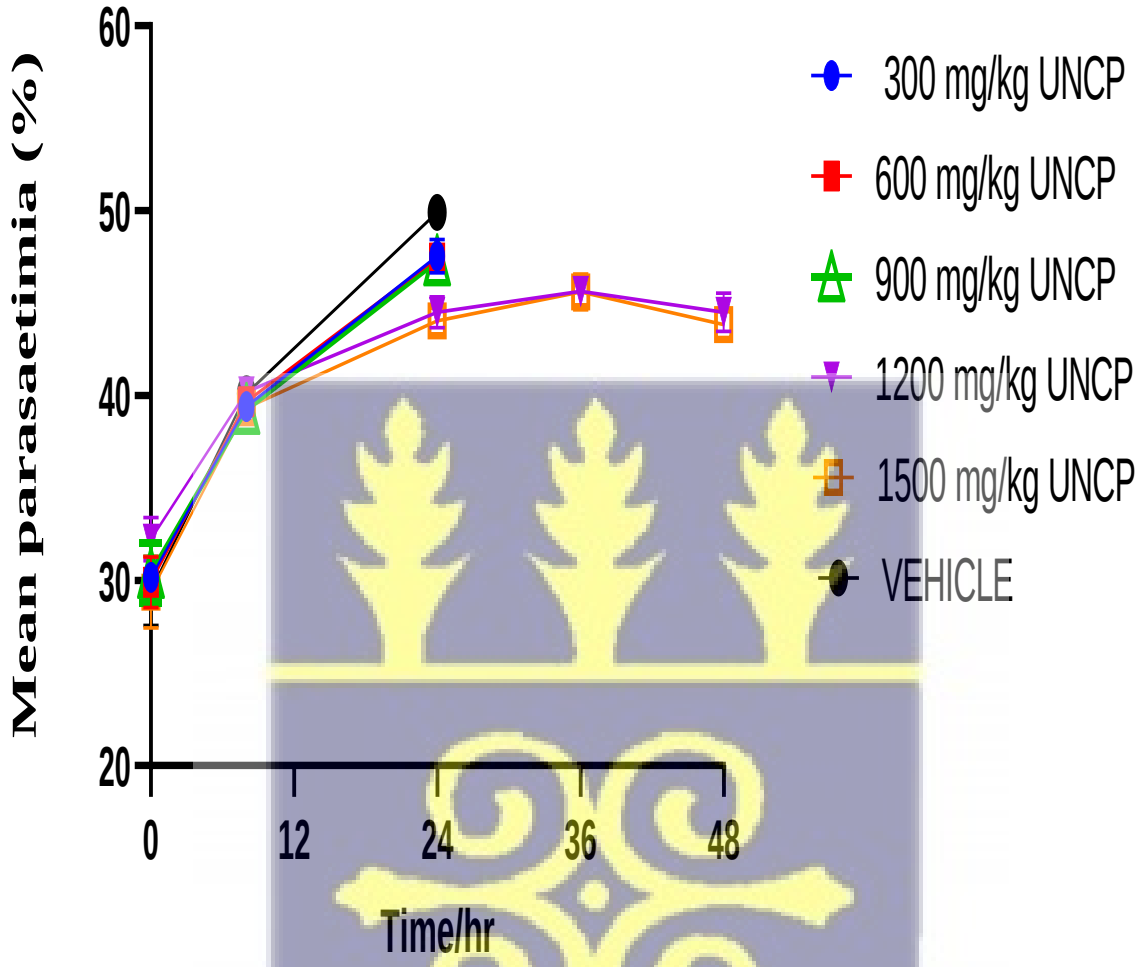


Figure 4.1: Time course graph of antimalarial effect of UNCP only

The varying doses of UNCP (300, 600, 900, 1200 and1500 mg/kg) on *Plasmodium berghei* (NK65) parasitaemia, last termination point on the curve represents the last blood sampling before subsequent animal deaths occurred.

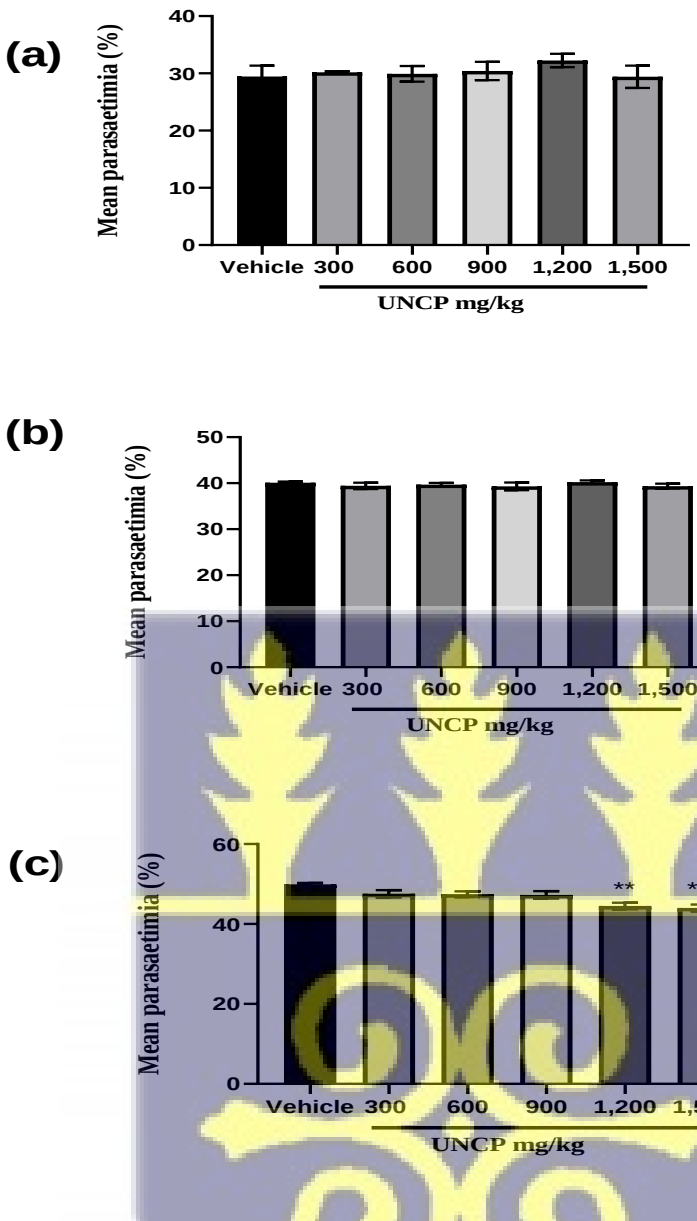


Figure 4.2: Antimalarial effect of UNCP only (1,500 and 1,200 mg/kg) at 0hr (a), 8hr (b) and 24hr (c)

*Values are mean ± SD (n = 5) and *P < 0.05, **P < 0.01 and ***P < 0.001 compared to control group (Two-way ANOVA followed by Bonferroni's multiple comparison* post hoc *analysis).*

4.6 UNCP+A/L CO-ADMINISTRATION GROUPS COMPARISON

Varying UNCP doses co-administered with a fixed dose of A/L, showed a general dose dependent decrease in parasitaemia with the increasing UNCP doses with total parasitaemia clearance obtained for all the groups in 60 hours except for the 300 mg/kg dose of UNCP for which total parasitaemia clearance was achieved at 72 hours (fig 4.3).

Even though there was an observable difference in parasite clearance, this was only significant at 36 and 48 hours of sample administration. A/L + UNCP (1,200 mg/kg) group showed significant parasite decrease with a percentage drop of 51.98% ($F_{6,28}$=1333, P < 0.0210) at 36 hours and in comparison, with the Coartem group recording a percentage drop of 39.63% (fig.4.4a). At 48 hours, A/L + UNCP (1,200 mg/kg) groups ($F_{6,28}$=1333, P < 0.0391) recorded a percentage drop of 71.3% in comparison with Coartem® only group with a percentage drop of 57.68% (fig.4.4b).

A/L + UNCP (1,500 mg/kg) group recorded the most significant parasite decrease ($F_{6,28}$=1333, P < 0.0001) in comparison with the Coartem® only group with a percentage drop of 55.89% and 39.63% respectively at time 36 hours (fig.4.5a) and percentage drop of 71.97% in comparison with Coartem® only group with a percentage drop of 57.68% at 48 hours (fig.4.5b).

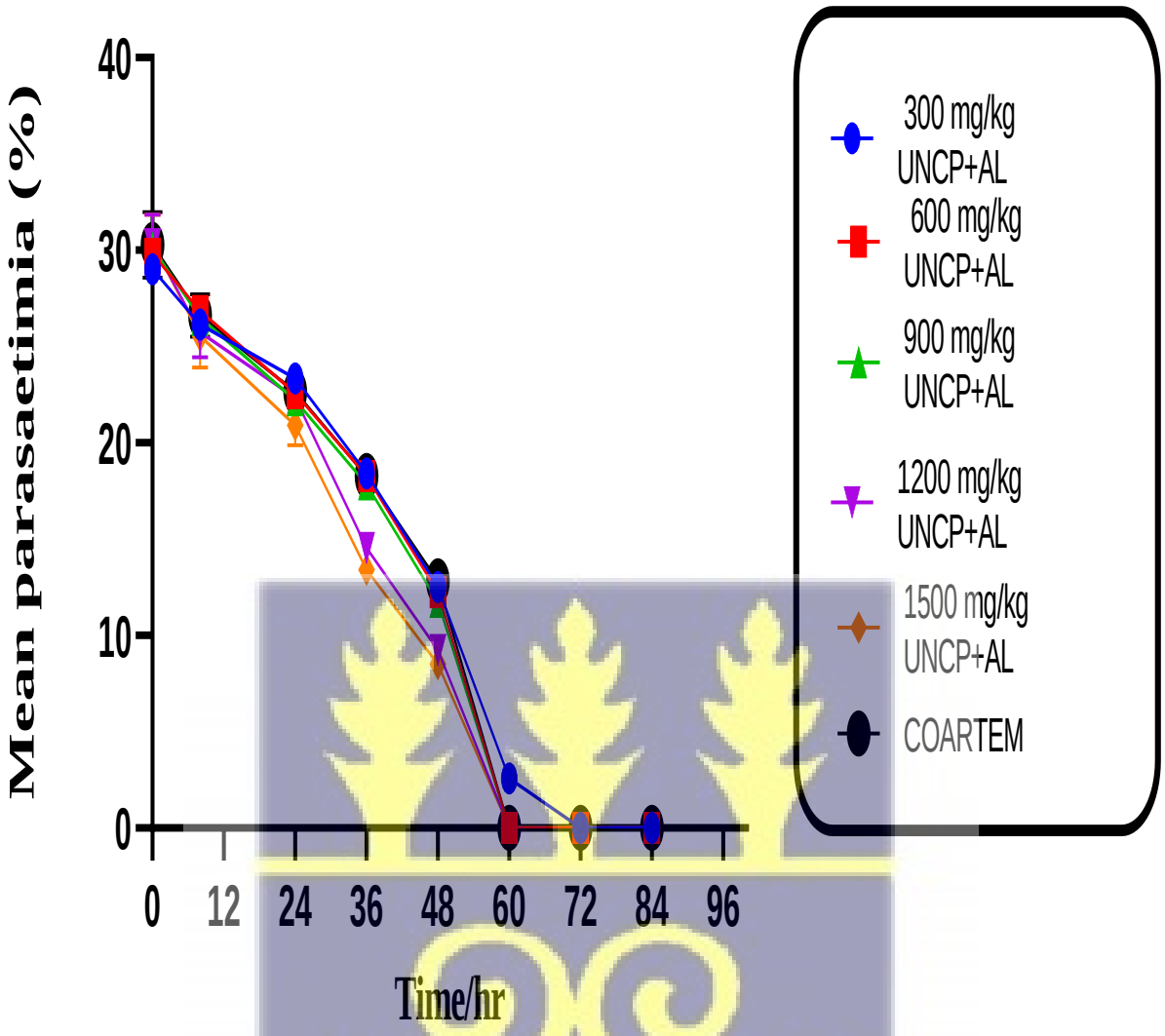
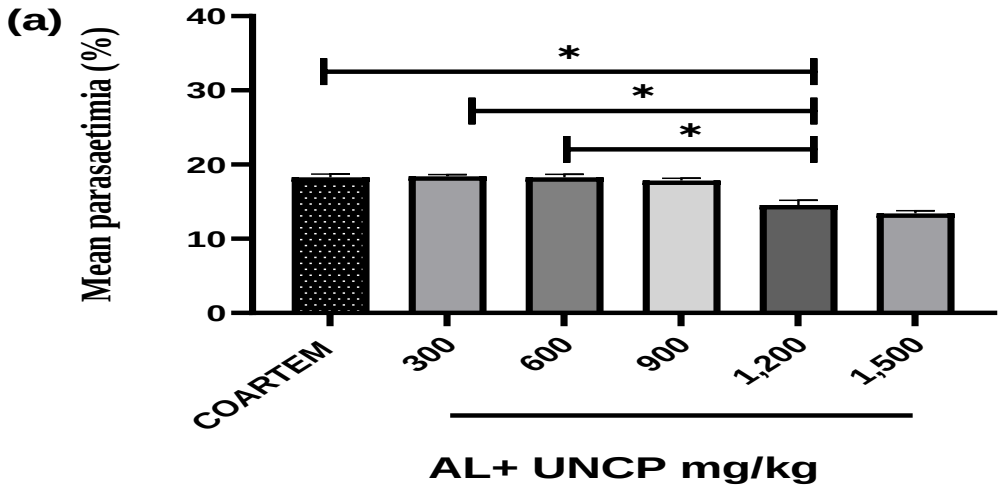


Figure 4.3: Time course graph of antimalarial effect of A/L+UNCP on *Plasmodium berghei* (NK65) parasitaemia.



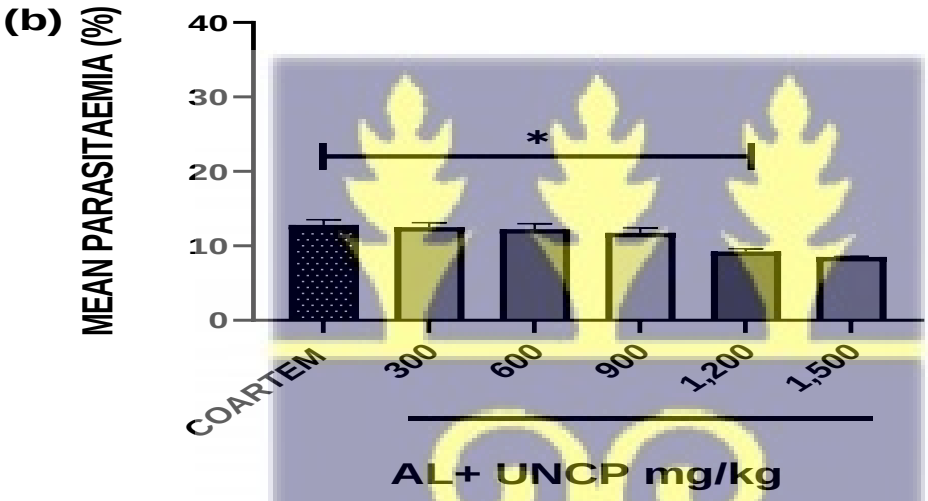


Figure 4.4: A 36-hour (a) and 48-hr (b) antimalarial effect of A/L+UNCP (1,200 mg/kg)

*Values are mean ± SD (n=5) and *P < 0.05, **P < 0.01 and ***P < 0.001 compared to control group (Two-way ANOVA followed by Bonferroni's multiple comparison post hoc analysis).*

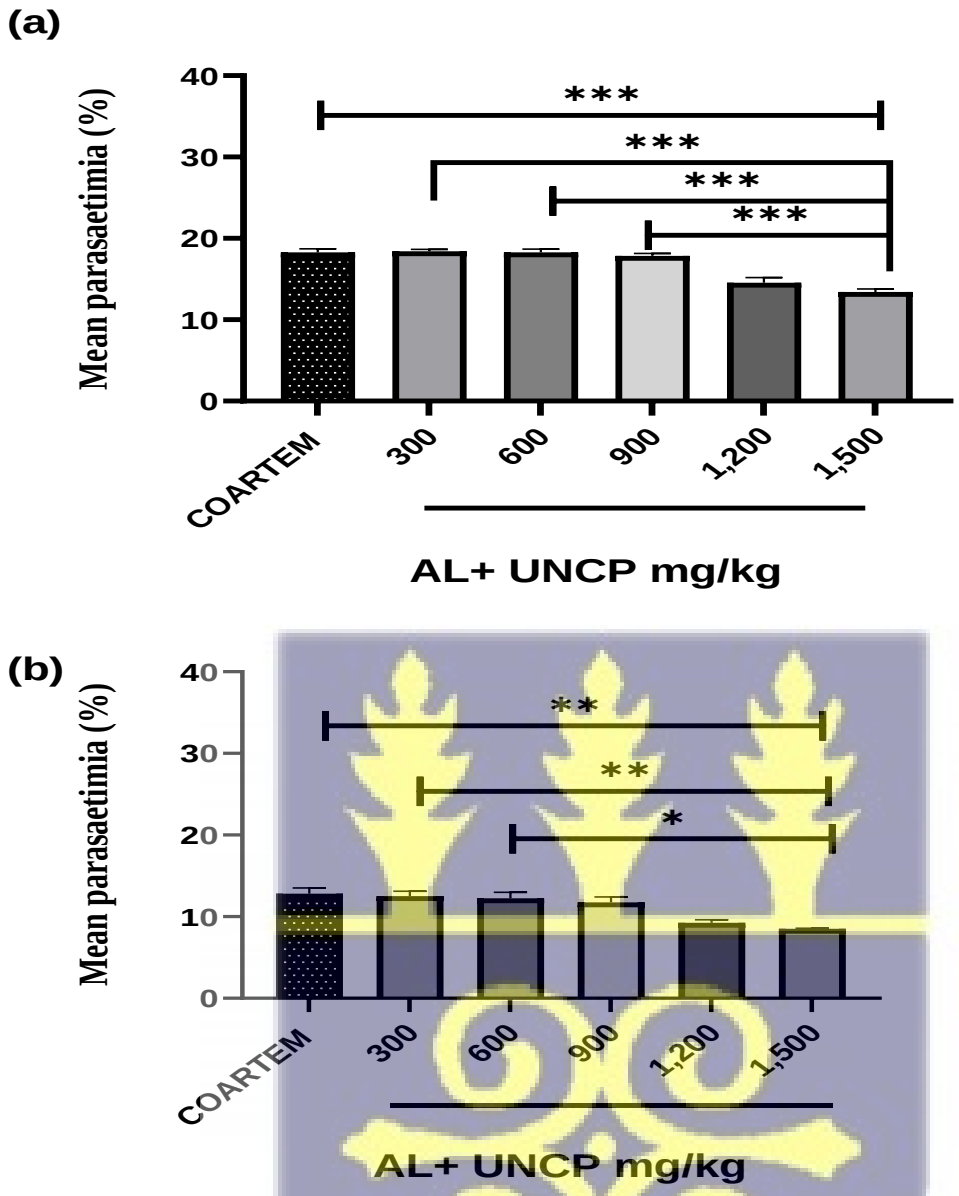


Figure 4.5: A 36-hour (a) and 48-hr (b) antimalarial effect of A/L+UNCP (1,500 mg/kg)

*Values are mean ± SD (n=5) and *P < 0.05, **P < 0.01 and ***P < 0.001 compared to control group (Two-way ANOVA followed by Bonferroni's multiple comparison post hoc analysis).*

4.7MEAN SURVIVAL TIME

Over a 28-day monitoring period, UNCP only groups had the least mean survival time (MST) with a range between 1-3 days/rats while the A/L+UNCP groups recorded the highest survival rates (27-28 days/rats) with no significant difference amongst these combination groups.

Table 4.3: Mean Survival Time of the various groups

GROUPS	MEAN SURVIVAL TIME (days/rats)
GROUP 1 – 300 UNCP (mg/kg)	1 ± 0.24
GROUP 2 – 600 UNCP (mg/kg)	1 ± 0.51
GROUP 3 – 900 UNCP (mg/kg)	1 ± 0.63
GROUP 4 – 1,200 UNCP (mg/kg)	2 ± 0.4
GROUP 5 – 1,500 UNCP (mg/kg)	2 ± 0.8
GROUP 6 – A/L + 300 UNCP (mg/kg)	27 ± 0.2
GROUP 7 – A/L + 600 UNCP (mg/kg)	27 ± 0.3
GROUP 8 – A/L + 900 UNCP (mg/kg)	27 ± 0.4
GROUP 9 – A/L + 1,200 UNCP (mg/kg)	28 ± 0.8
GROUP 10 – A/L + 1,500 UNCP (mg/kg)	28 ± 0.9
GROUP 11 – (DISTILLED WATER)	1 ± 0.1
GROUP 12 COARTEM® (mg/kg)	27 ± 0.8

4.8 HPLC ANALYSIS OF LUMEFANTRINE (PHASE TWO)

Calibration Curve of Lumefantrine

The calibration curve was obtained using calibration samples of lumefantrine standard concentration range of 0.05- 0.5 ng/µl (Appendix 2). The result indicated that the concentration X of lumefantrine standard and the peak area Y showed a good linearity. Regression analysis yielded the equation: y = 54049.51430x +172.93341 (R^2 = 0.99977), standard deviation of 235.26825.

Table 4.4: Calibration table of Lumefantrine standard concentrations and their corresponding areas and Rsp Factors.

LUMEFANTRINE STANDARD	CONC (ng/µl)	AUC	RSP. FACTOR
STANDARD 1	0.05	360.78470	$1.38587e^{-4}$
STANDARD 2	0.1	750.04858	$1.33325\ e^{-4}$
STANDARD 3	0.2	1508.87183	$1.32549\ e^{-4}$
STANDARD 4	0.3	2150.29126	$1.39516\ e^{-4}$
STANDARD 5	0.4	2872.52393	$1.39250\ e^{-4}$
STANDARD 6	0.5	3588.31055	$1.39341\ e^{-4}$

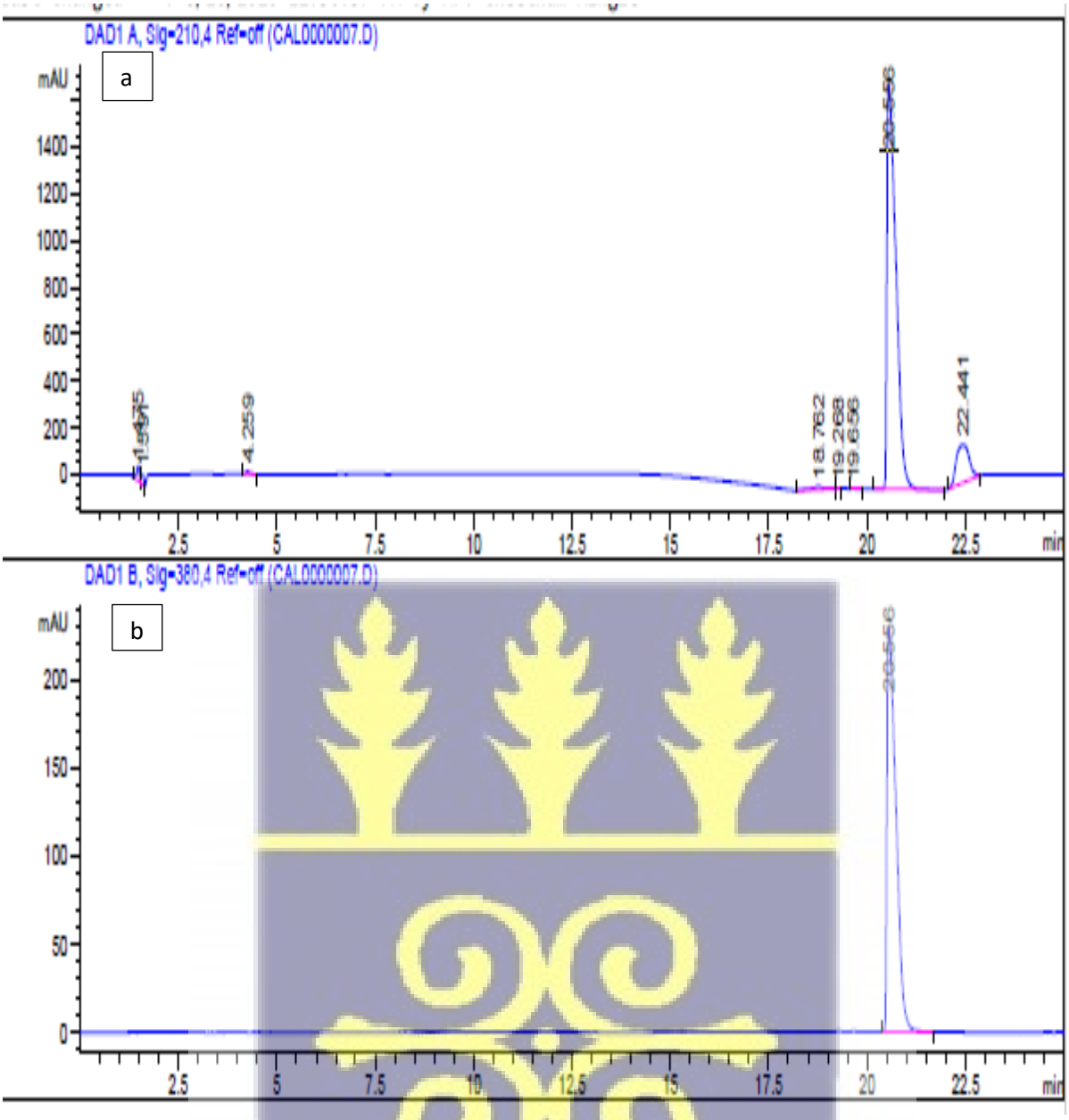


Figure 4.6: Chromatographic peaks of standard lumefantrine

UV-Vis wavelengths of 210nm and 380nm as shown in (a) and (b) respectively at a retention time of 21 minutes.

A plasma concentration curve for lumefantrine in conjunction with varying doses of UNCP showed a general increase from time zero with a steep rise occurring within 4 – 8 hours followed by a general decrease within 24 hours post dose administration.

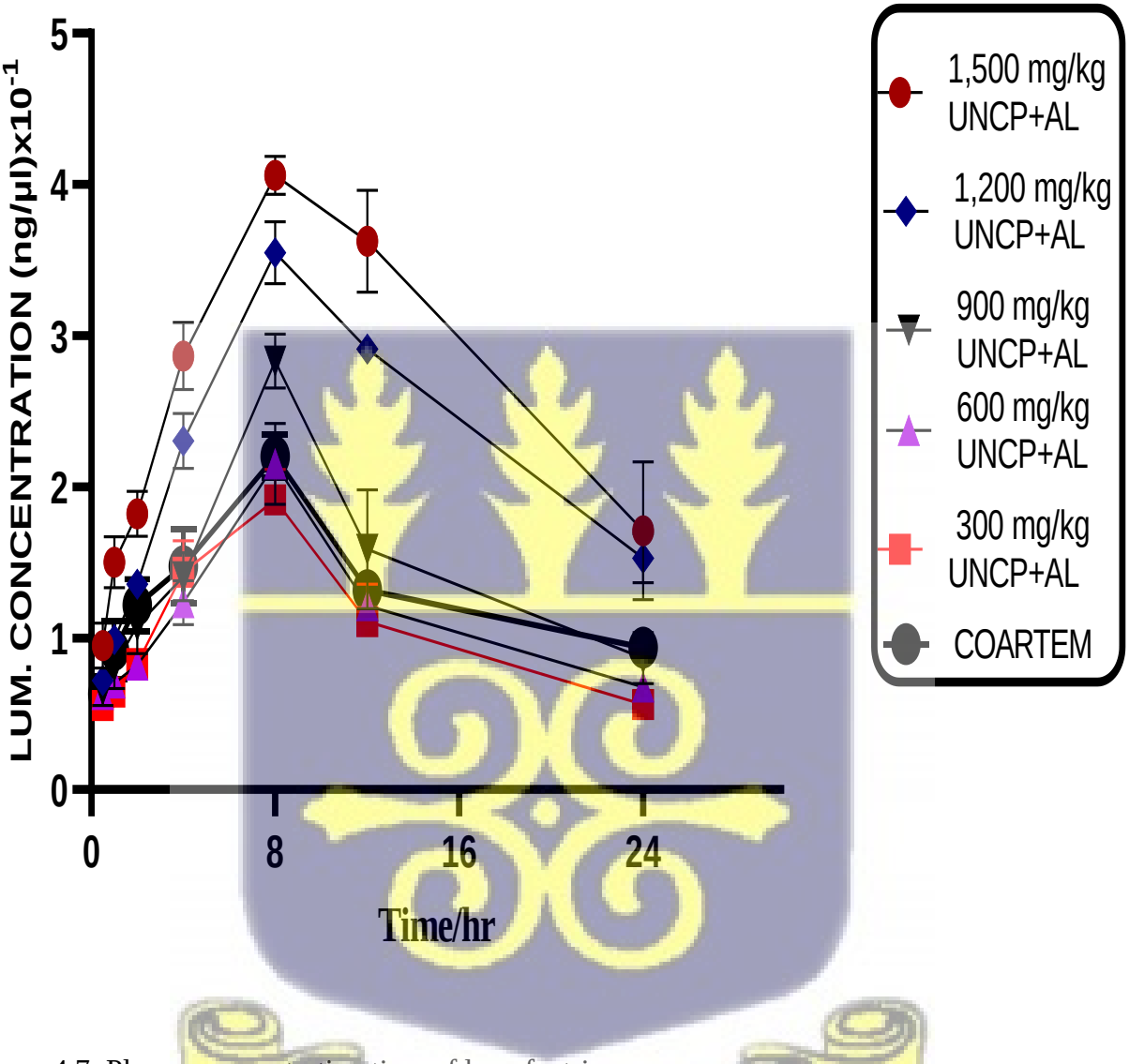


Figure 4.7: Plasma concentration time of lumefantrine

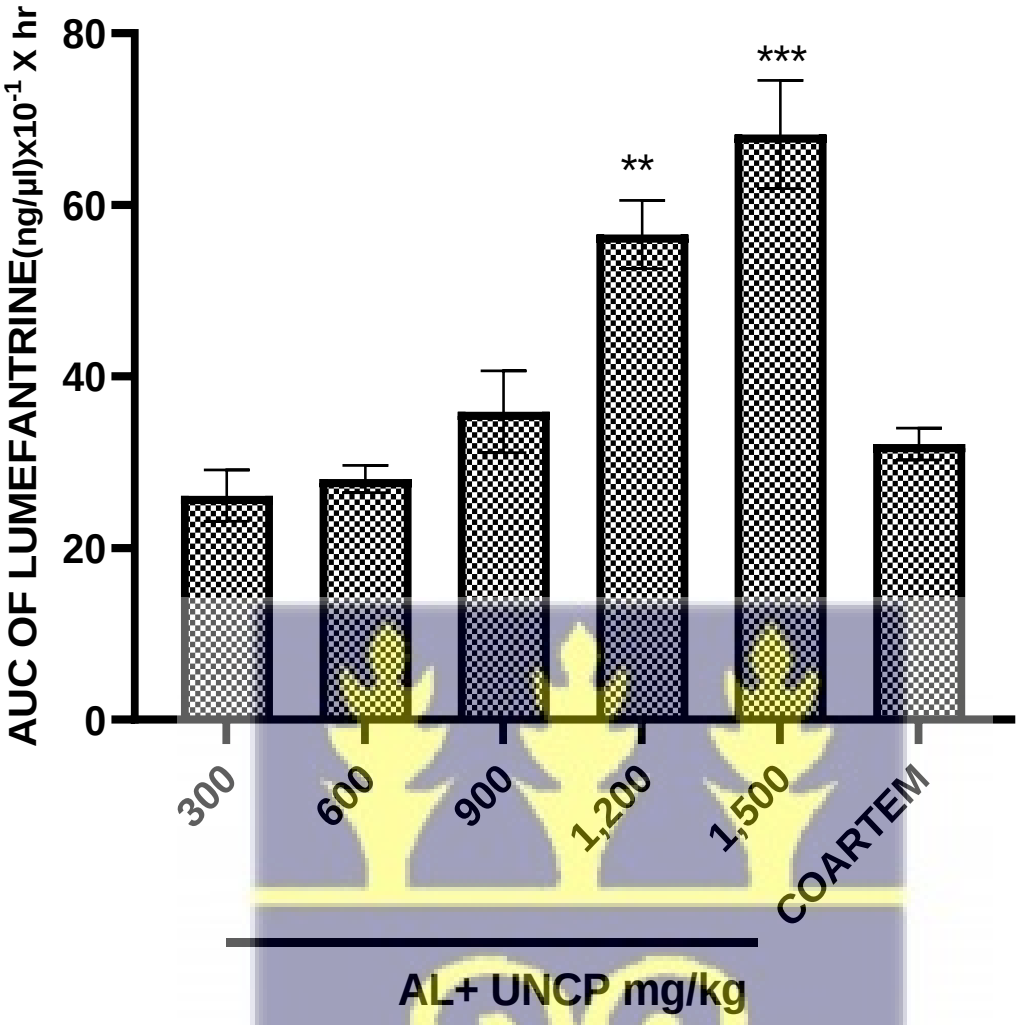


Figure 4.8: AUC of lumefantrine in combination with various doses of UNCP

*Values represented as AUC (n = 3) and *P < 0.05, **P < 0.01 and ***P < 0.001 compared to control group (One-way ANOVA followed by Bonferroni's multiple comparison post hoc analysis).*

Table 4.5: Results of Pharmacokinetic Summary

PK Parameter	Treatment Group [Mean ± SEM]						p-value
	A/L +300 mg/kg UNCP	A/L +600 mg/kg UNCP	A/L +900 mg/kg UNCP	A/L +1,200 mg/kg UNCP	A/L +1,500 mg/kg UNCP	ONLY COARTEM	
T_{max} (hr)	8.170± 0.03512	8.103± 0.02906	8.307± 0.1244	8.103± 0.04096	8.277± 0.03480	8.190± 0.05033	0.1667
C_{max} (ng/µl*10^{-1})	1.917± 0.1994	2.153 ± 0.2686	2.833± 0.1770	3.550± 0.2066	4.060± 0.1266	2.203± 0.1431	P<0.0001
$AUC_{0\rightarrow24}$ (ng/µl*10^{-1}*hr)	26.11± 3.009	28.04± 1.590	35.89± 4.786	56.57± 2.132	68.21± 6.114	32.12± 1.853	P<0.0001
K_e (hr^{-1})	0.05281± 0.01940	0.04880± 0.002344	0.04873± 0.005647	0.05466± 0.009233	0.06813± 0.01649	0.02933± 0.007221	0.3879
$t_{1/2}$ (hr)	17.75± 0.1994	14.27± 0.2686	14.65± 0.1770	13.26± 0.2066	11.86± 0.1266	26.23± 0.1431	0.2002

Results of Liver Function Test

Table 4.6: Liver biochemical analysis for the various groups

LIVER FUNCTION TEST	A/L +300 mg/kg UNCP	A/L +600 mg/kg UNCP	A/L +900 mg/kg UNCP	A/L +1,200 mg/kg UNCP	A/L +1,500 mg/kg UNCP	COARTEM ONLY	VEHICLE ONLY	p-value
AST U/L	478.20 ± 47.02	497.60 ± 39.90	624.32 ±187.35	483.90 ± 33.38	564.52 ±194.41	774.16 ±198.61	863.01 ±117.6	0.3790
ALT U/L	99.02 ± 23.62	104.82 ± 20.41	204.42 ± 43.76	187.88 ±25.07	263.04± 92.58	278.48 ±44.51	204.9 ±23.78	0.1962
ALP U/L	119.14 ±32.22	131.04 ±35.33	188.72 ±25.46	118.04 ±24.07	204 ±49.94	305.8 ±13.47	225.34 ±15.20	0.0043
ALBUMIN g/mL	67.58 ±20.34	70.12 ±23.29	159.4 ±53.88	56 ±18.65	200.48 ±110.74	200.48 ±46.25	60.8±15.81	0.3495

CHAPTER 5

DISCUSSION

Amongst the UNCP only groups, there was an initial general increase in parasitaemia throughout the entire time frame with a steeper rise amongst the lower UNCP only doses than the higher doses (1,200 and 1,500 mg/kg) as shown in Fig. 4.1. However, this was only significant within 24 hours for the higher doses of the UNCP i.e. 1,500 mg/kg ($F_{6,28}$=459, P <0.0001) and 1,200 mg/kg $F_{6,28}$=459, P < 0.0029) compared to the vehicle group as shown in Fig.4.2 (c).This observation is suggestive of the presence of parasitaemia suppressing agents in the UNCP which is in line with other studies with the observed antimalarial activity being attributed largely to the presence flavonoids and other key phytochemicals such as alkaloids and tannins (F. J. M. h. Addai, 2010; Amponsah et al., 2012; Bankole et al., 2016; Fasola, Iyamah, Sciences, & Technology, 2014).

Flavonoids are one of the most ubiquitous phytoconstituents in our daily plant diets, the antimalarial activity of flavonoids has been reported by several studies such as, Lim *et al.* (2007) which reported the antimalarial properties of key flavonoid derivatives. Other studies by Mobrison *et al.* (2006) and Al-droey *et al.* (2011) have all demonstrated a strong relationship between the antimalarial activity and flavonoid content and these corroborate the findings from our study. This occurs through the inhibition of fat biosynthesis on the malaria parasite through the inhibition of ingress of L-glutamine and myoinositol. Another possible mechanism as shown in literatures is the targeting the -OH groups of important biomolecules such as DNA and proteins resulting in the formation of radical anions causing oxidative stress and damage to the plasmodium parasite (Ferreira, Luthria, Sasaki, & Heyerick, 2010; Graham et al., 2019). Some dietary flavonoids found to possess antimalarial activity include acacetin, biacaclein, chrysin, isoquercetin, and quercetin (de Monbrison et al., 2006; Khaomek et al., 2008; Lim, Kim, & Lee, 2007).

Another phytochemical group that possesses antimalarial activity are the alkaloids. Studies show the ability of alkaloids to inhibit the growth of *P. berghei in vivo* and a higher survival rate with respect to the presence of beta-carboline alkaloids (Ang et al., 2000). In vitro studies also demonstrate antimalarial activity of specific alkaloids such as 6-hydroxyhaemanthanine, haemanthanine, photoberberine and lycorine which are potent against *P. falciparum* (Ancolio et al., 2002; Iwasa, Kim, Wataya, & Lee, 1998).

Apart from phytochemicals that have demonstrated a strong correlation with antimalarial activity, the presence of significant amounts of trace minerals such as manganese, zinc and copper have been shown to possess an immunostimulatory effect and consequently impedes the progression in parasitaemia (F. K. Addai, 2010; Jayeola, Oluwadun, Olubamiwa, Effedua, & Kale, 2011). A previous study performed on our UNCP sample by Asiedu-Gyekye *et al.* (2016) has reported the presence of 12 macroelements and 26 microelements including manganese, zinc and copper. Zinc plays a central role in the development of the immune system via a host of different mechanisms including development and functioning of immune cells such as neutrophils and natural killer cells. It also regulates the growth and modulation of the acquired immunity such as T lymphocytes and B lymphocytes, and immune mediators like enzymes, cytokines and thymic peptides (Dardenne, 2002; Ibs & Rink, 2003). Apart from zinc, there is evidence of copper playing an important role in the immune system, studies have shown the link between the reduction of interleukin 2 and copper deficiency which helps in T cell proliferation (Bonham, O'Connor, Hannigan, & Strain, 2002). Thus, the presence of these key elements in UNCP may have contributed to its antimalarial activity and these immune mechanisms may provide possible explanation to the higher survival rates for the 1,200 and 1,500 UNCP groups.

The results showed that 1,200 mg/kg and 1,500 mg/kg UNCP only groups survived 24 hours longer than the other groups with a mean percentage parasitaemia of 47.3% and 46.1% respectively and their mean survival time of 2 ± 0.4 days/animals and 2 ± 0.8 days/animals respectively (Table 4.7). MST is a parameter which gives a good indication of the average survival length per animal in examination of the effect of a test sample administered (Royston & Parmar, 2013; Royston & Parmar, 2011). It was observed that the low dose UNCP only groups (300-900 mg/kg UNCP) recorded the least MST which may be suggestive that higher doses are needed to stimulate enough immune response against the malaria parasite. These findings about UNCP could provide a new insight into its role as both a prophylactic and adjunct dietary agent in the clinical management of uncomplicated malaria.

When varying UNCP doses were co-administered with a fixed dose of A/L, a general dose dependent decrease in parasitaemia was observed with the increasing UNCP doses (fig 4.3) with total parasitaemia clearance obtained for all the groups in 60 hours except for the 300 mg/kg dose of UNCP for which total parasitaemia clearance was achieved at 72 hours.

Even though there was an observable difference in parasite clearance, this was only significant within 36 hours and 48 hours for A/L + UNCP (1,500 mg/kg) and A/L + UNCP (1,200 mg/kg) groups. It was observed that A/L + UNCP (1,200 mg/kg) showed significant parasite decrease (*P < 0.0210) in comparison with the Coartem® only group with a percentage drop of 51.98% and 39.63% respectively. An intergroup comparison also revealed similar trend with A/L + UNCP (1,200 mg/kg) showing significance (*P < 0.0240) when compared with A/L + UNCP (300 and 600 mg/kg) at the same time. Also, at 48 hours, A/L + UNCP (1,200 mg/kg) showed significant parasite decrease compared to the Coartem® group (*P < 0.0391) with a percentage drop of 71.3% and 57.68% respectively for the A/L + UNCP and Coartem®. Results also indicate that AL +

UNCP (1,500 mg/kg) group recorded the most significant parasite decrease (***$P < 0.001$) in comparison with the Coartem® only group with a percentage drop of 55.89% and 39.63% respectively within 36 hours and a percentage drop of 71.97% (**$P < 0.0017$) and 57.68% respectively within 48 hours.

According to the Regan Shawn *et al. 's* (2008) dose calculation model, UNCP doses of 1,500 mg/kg and 1,200 mg/kg is equivalent to 4 and 3 teaspoons of human doses respectively. This can be recommended in addition to malaria treatment especially in children below five years, an extremely vulnerable group with inadequate immune system. A research by the WWARN on a 28-day PCR-therapeutic efficacy study of A/L indicates that therapeutic efficacy was lowest in young malnourished children underweight children in Africa with age range of 1-3 years (Reagan-Shaw, Nihal, & Ahmad, 2008; WWARN, 2015). This group have always borne the heaviest malaria burden and accounts for about 60% of total malaria deaths worldwide which is quite alarming. Over the years, few research efforts have been channelled towards this group. One of such is the development of malaria vaccine pioneered under the auspices of WHO which when successful will be a major breakthrough in our fight against malaria. As the development of vaccine is quite sophisticated, expensive and may take years, other research avenues may be needed to diversify our approach in the fight against malaria. One of such approaches is the exploration of our phytotherapy. Africa contributes over 70% of the total global production of cocoa which includes countries such as Ghana, Ivory Coast, Nigeria and Cameroun making UNCP readily and cheaply available in Africa. A treatment strategy incorporating UNCP into A/L malaria management in Africa is likely to afford more cost-effective treatment and therapeutic outcomes and some suggestions have been made that these nutraceuticals may not only be supplementary in malaria treatment by may even have prophylactic roles (Calkins & Ngo, 2005; Lundstedt & Pärssinen,

2009; Wessel & Quist-Wessel, 2015). Alternatively, UNCP, may be explored as a possible excipient in the preparation of A/L tablets and powders meant for reconstitution into suspensions for paediatric use.

A/L as an antimalarial agent used in the treatment of uncomplicated falciparum malaria is fraught with the problem of poor GI absorption and this improves with the presence of fat when the drug is administered with fatty food (Awofisayo, Arhewoh, & Okhamafe, 2019; Borrmann et al., 2010; Ezzet et al., 2000; Premji et al., 2008). UNCP has been found to a rich in fat content of about 28% as shown in Table 5 which may be able to aid the absorption of A/L. The second phase of the study sought to investigate this property. The pharmacokinetic model employed was a non-compartmental model which considers the test subject as one homogenous compartment This approach has been used in numerous studies as it is efficient and effective (Diao & Meibohm, 2013; Gabrielsson & Weiner, 2012). The HPLC-UV/Vis method used sought to detect the presence of both Artemether and Lumefantrine, however, only the latter was detected which could be due to factors such as; lack of chromophores in artemether and low proportion in comparison with lumefantrine (dose ration of 1:6) which has been observed in numerous studies (Fabre, Blanchin, Montels, & Aké, 2013; Huang et al., 2010). A study by Huang *et al* (2010) reported a similar challenge in the effort to simultaneously identify both artemether and lumefantrine, this was also reported by Fabre *et al* (2013) who proposed the use capillary electrophoretic method- a separation technique of constituents based on their electric charge which have proved to be more efficient, effective with low sample injection needed. Notwithstanding, the lumefantrine results obtained in this study gives a good account of the effect of UNCP on A/L absorption, this is because amongst the two agents, the influence of fat is higher in lumefantrine (sixteen fold) than artemether (two-fold) due to the high lipophilicity of the former, hence easily partitioned in fats to aid in absorption.

The HPLC chromatograph for lumefantrine showed good symmetric peaks at wavelengths of both 210 nm and 380 nm with a retention time of 21 minutes. A concentration time-course graph showed a general rise of lumefantrine concentrations for the various groups which peaked at 8 hours post administration with a slow decline observed for A/L+UNCP (1,200 and 1,500 mg/kg) but a sharp fall for the other groups (fig.4.7)

The influence of food has been established as an important factor in drug absorption in most cases either increasing or decreasing and in some cases causing the delay in gastric emptying time and intestinal transit time. The AUC of a drug gives an indication of the extent of absorption of the drug. The results of the study showed a higher AUC for A/L+UNCP (1,200 mg/kg) and A/L+UNCP (1,500 mg/kg) groups (**$P < 0.0091$) and (***$P < 0.0003$) in comparison with the Coartem® group respectively. This translates into an increment of 43.22% and 52.97% for AL+UNCP (1,200 mg/kg) and AL+UNCP (1,500 mg/kg) groups respectively higher than the Coartem® only group. This finding shows the possible influence of UNCP and its fat content (28%) in aiding the absorption of lumefantrine. This is in agreement with a study by Mwebaza *et al* (2013) which examined the comparable oral bioavailability of lumefantrine co-administered with oil-fortified maize porridge and milk with results indicating that there was a higher bioavailability of in these groups in contrast with fasted individuals taking only the drug. Also, a research by Ashley *et al* (2007) in the determination of the amount of fat in soya milk needed to optimize oral lumefantrine absorption showed that a 36 ml of milk corresponding to 1.2g of fat was needed to achieve 90% of maximum effect (in terms of AUC).

The peak time (Tmax) of a drug gives an indication of the time taken to achieve maximum plasma concentration, the longer the Tmax the longer it takes for a drug to reach its maximum concentration (Cmax); at which point, the rate of absorption is the same as the rate of elimination

(Ezzet et al., 2000). Tmax can also be used in assessing the bioavailability, in choosing the preferred route of administration and to determine the onset of action of a drug (Tarning et al., 2009). The Tmax results obtained amongst the groups showed no significant difference, this shows that UNCP of various doses did not have any significant effect on the time taken by A/L to reach its maximum concentration.

On the other hand, comparison of the peak concentration (Cmax) of the various groups showed a 37.9% higher concentration for A/L+UNCP (1,200 mg/kg) in comparison with the Coartem® group (**$P < 0.0051$). The results also indicate a similar trend for A/L+UNCP (1,500 mg/kg) which showed a 45.7% higher concentration (***$P < 0.0003$) than the Coartem® group with a mean peak concentration. The results build on existing evidence of the influence of food on the absorption of A/L especially lumefantrine.

The liver becomes the most vital organ of interest in this study because; not only does it serve as the site for the hepatic stage of the malaria parasites life cycle but also a place for A/L metabolism thus prone to injury (Mwebaza et al., 2013; Owumi et al., 2015). The LFT results showed significant increase of ALP (**$P < 0.0043$) for only AL+UNCP (300 mg/kg) group; since the LFT profile was consistent for almost the entire study, high serum ALP concentration for this group could be from sources other than the liver such as bone and kidney, nevertheless future investigations may be needed to draw a firm conclusion. Research indicates that high doses of A/L may lead to cardiac, hepatic and renal damage (Owumi et al., 2015). A study by Asiedu-Gyekye et *al* (2016) found UNCP to attenuate high doses of A/L toxicity to these organs.This shows the cruciality of UNCP not only in terms of its antimalarial and bioavailability contribution but also ameliorating A/L-induced organ toxicity even in rare cases of overodose and safeguarding the patient.

CONCLUSION

The findings from the study showed that high doses of UNCP (1,200 mg/kg and 1,500 mg/kg) co-administered with A/L gave a better parasite clearance than A/L alone. Also, these UNCP doses also gave a higher lumefantrine plasma concentration in contrast with A/L alone with these co-administrations showing a safe hepatic profile. These above UNCP doses could be recommended as a dietary adjunct in the clinical management of uncomplicated malaria to improve A/L treatment outcomes.

LIMITATION

The study sought to determine the plasma concentration for both artemether and lumefantrine as both were administered, however, only lumefantrine analysis was possible which would have given a better view in terms of the influence of fat food presence in UNCP on the extent of gastrointestinal absorption of A/L.

RECOMMENDATION

1.It is recommended, other analytical methods such as electrophoresis, HPLC/MS to be used to help ascertain the effect of food on artemether.

2. Histopathological studies should be performed on the liver as the LFT results even though very useful, may not give an indication of the entire extent of the liver damage.

3. Higher doses of UNCP can be examined to help determine the safety and optimum dose limit.

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APPENDICES

Appendix 1: Template of Dosing Schedule for AL/UNCP Co-administration for Pharmacokinetic Study

TIME / ANIMALS PER GROUP	EVENING	MORNING	EVENING	MORNING
	0 HR	12 HRS	24HRS	36HRS
A1	6:00pm	6:00am	6:00pm	6:00am
A2	6:10pm	6:10am	6:10pm	6:10am
A3	6:20pm	6:20am	6:20pm	6:20am

Appendix 2: Calibration Curve of Standard Lumefantrine at Various Concentrations

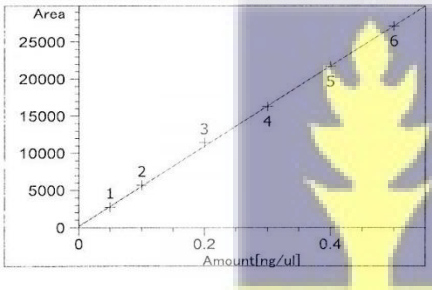
Method C:\Chem32\1\METHODS\PRINCE.M

RT	Sig	Lvl	Amount [ng/ul]	Area	Rsp.Factor	Ref	ISTD #	Compound
		6	5.00000e-1	2.71437e4	1.84205e-5			
19.944	2	1	5.00000e-2	360.78470	1.38587e-4			
		2	1.00000e-1	750.04858	1.33325e-4			
		3	2.00000e-1	1508.87183	1.32549e-4			
		4	3.00000e-1	2150.29126	1.39516e-4			
		5	4.00000e-1	2872.52393	1.39250e-4			
		6	5.00000e-1	3588.31055	1.39341e-4			

Peak Sum Table

No Entries in table

Calibration Curves



LUMIFANTHRINE at exp. RT: 19.944
DAD1 A, Sig=210,4 Ref=off
Correlation: 0.99977
Residual Std. Dev.: 235.26825
Formula: y = mx + b
m: 54049.51430
b: 172.93341
x: Amount
y: Area

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Appendix 3: Ethical Clearance Letter

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES

ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/MAR/2020 March 11, 2020

Mr. Akotuah Prince Appiah
Department of Pharmacology and Toxicology
School of Pharmacy
Legon

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M5 –5.3/2019-2020***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) on March 11, 2020 unanimously reviewed and approved your re-submitted research protocol.

Title of Protocol: **"Evaluating the Malaria Parasite Clearance and Pharmacokinetics of Artemether Lumefantrine Co-Administered with Unsweetened Natural Cocoa Powder"**

Principal Investigator: Mr. Akotuah Prince Appiah

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till March 12, 2021.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, SOP
Head, Department of Pharmacology and Toxicology

• P. O. Box KB 52, Korle Bu, Accra, Ghana. • Telephone: +233 (0) 306 665103/4 • Fax: +233 (0) 302 660762
• Email: admin.chs@ug.edu.gh / provost.chs@ug.edu.gh • Website: www.chs.ug.edu.gh

Appendix 4: Results of Total Fat Assay of UNCP

TITLE: Analytical Test Report

Our Ref: 09/NFS/FL/20

TO:

LABORATORY CONDUCTING TEST

Food Laboratory

Nutrition and Food Science Department

University of Ghana

P. O. Box LG 134

Legon

NAME OF SAMPLE: Brown Gold Natural Cocoa Powder

DATE RECEIVED: 25/06/2020 **DATE OF PERFORMANCE:** 6/07/2020

PURPOSE: Quality Evaluation

TEST CONDUCTED	UNIT	RESULT
Fat content	g/100g	28.11

SIGNATURE: ..... **SIGNATURE:** .....

REPORTED BY: JOYCE DUAH **APPROVED BY:**

(ANALYST) (HEAD OF DEPARTMENT)

DATE: 8/7/2020 **DATE:** 8/7/2020