INVESTIGATING THE MODULATORY EFFECT OF ARTEMETHER/LUMEFANTRINE ON HEPATIC CYP450 ENZYME IN ALBINO RATS

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FACULTY OF PHARMACY

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FUFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHARMACY (PHARM.D.) DEGREE

NOVEMBER, 2025

CERTIFICATION

We the undersigned hereby agree that this work was carried out by IDOJEH AGHOGHO GOODLUCK with matriculation number PHA1908513 in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City.

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DEDICATION

I dedicate this work to Almighty God and in loving memory of my parents, late Miss Comfort Enagemo and Mr. Spencer Idojeh, whose love lives within me.

ACKNOWLEDGEMENTS

I am profoundly grateful to God for His continued providence, grace, and unwavering aid throughout my academic journey and this project.

My sincere appreciation goes to my project supervisor, Prof. S.E.O. Aghahowa for his invaluable support, mentorship and guidance.

My deepest appreciation goes to my late parents, Miss Comfort Enagemo and Mr. Spencer Idojeh. Though you are no longer here, your love and prayers still guide my path. This achievement is as much as yours as it is mine.

To my siblings, who have stood as second parents in every sense: Mrs. Linda Ejovwoke Ovie, Miss Ighofose Blessing Iroro, Mr. Akpofure Clinton Idojeh, Barr. Eferurhobo Akpobome, Prince; your love and strength have carried me through.

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ABSTRACT

Background: Artemether/Lumefantrine (AL) is one of the most widely used artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated malaria. Given its extensive use in Nigeria and globally, a proper understanding of its drug-drug interaction potential is essential. A key mechanism underlying such interactions is the regulation of hepatic Cytochrome P450 (CYP450) enzymes. However, there is a pharmacological paradox: artemisinin derivatives such as artemether are often reported as enzyme inducers, whereas lumefantrine has been associated with inhibitory effects. It remains unclear what net effect the AL combination exerts on CYP450 expression, and this knowledge is critical for predicting clinical outcomes.

Aim: The aim of this study was to examine the potential inductive or inhibitory action of AL on hepatic CYP450 enzymes in albino rats.

Methods: Twenty-four albino rats were randomly divided into four groups (n=6): Group A (Olive oil vehicle control), Group B (Cimetidine inhibition control), Group C (Phenobarbital induction control), and Group D (AL test group). Treatments were administered orally once daily for 14 days, with AL given twice daily to reflect its clinical regimen. On day 15, animals were sacrificed and livers harvested for microsomal preparation. Microsomal protein concentration was determined using the Modified Lowry method, while total CYP450 content was quantified spectrophotometrically using the reduced carbon monoxide (CO) difference method.

Results: The normalized CYP450 content (nmol/mg protein) of AL (0.165 ± 0.020) was not significantly different from the olive oil control (0.204 ± 0.022) but was significantly lower than cimetidine (0.451 ± 0.069) and phenobarbital (0.717 ± 0.186). This indicates that the expected substrate-driven induction was nullified in the AL group, aligning its CYP450 content with the inhibitory baseline. There was no significant difference in the percentage weight change between the AL group (20.10 ± 1.85) and the control group (14.20 ± 4.18). Supporting parameters such as protein concentration (16.87 ± 2.20) and denatured protein (37.70 ± 0.03) confirms that AL's effect was specific to CYP450 modulation and not due to systemic toxicity.

Conclusion: This study shows that Artemether/Lumefantrine (AL) is a net hepatic CYP450 enzyme inhibitor in albino rats. Clinically, AL may slow the metabolism of co-administered drugs, increasing the risk of accumulation and toxicity in polypharmacy cases common in malaria-endemic regions. This highlights the need for careful monitoring and possible dose adjustments of concomitant medications.

Keywords: Artemether-Lumefantrine, Artemisinin-Based Combination Therapy (ACT), Cytochrome P450, Drug-Drug Interactions, Enzyme Inhibition, Rats, Malaria Chemotherapy.

CHAPTER ONE

1.1 Malaria

1.1.1 Incidence and Distribution

Malaria remains one of the most persistent and devastating parasitic diseases worldwide, with its highest burden concentrated in tropical and subtropical regions (World Health Organization [WHO], 2024). Incidence refers to the number of new malaria cases occurring in a defined population over a specific time period, typically expressed per 1,000 individuals at risk. Monitoring incidence is critical for assessing the effectiveness of control measures, identifying vulnerable populations, and guiding the allocation of resources (Centers for Disease Control and Prevention [CDC], 2024).

In 2023, global malaria cases increased compared to 2022, signaling a slowdown in the progress achieved since the early 2000s. This stagnation is attributed to multiple converging factors, including insecticide resistance, emerging antimalarial drug resistance, climate variability, and health system disruptions (WHO, 2024). Nearly half of the world's population remains at risk, with the WHO African Region bearing the greatest share of both cases and deaths. This disproportionate burden is driven by the dominance of *Plasmodium falciparum* and the presence of highly efficient mosquito vectors such as the *Anopheles gambiae* complex (Bhatt *et al.* 2021).

Table 1.1 below summarises the regional malaria landscape:

Region	**Transmission Pattern**	**Predominant Species**	**Snapshot Notes**
Sub-Saharan Africa	Perennial, high intensity	*P. falciparum*	Highest incidence and mortality; many areas >200 cases/1,000 at risk
South-East Asia	Seasonal, heterogeneous	*P. falciparum*, *P. vivax*	Large case counts; uneven progress
Eastern Mediterranean	Focal, instability-affected	*P. falciparum*	Conflict and poor access sustain high incidence
Americas	Focal (Amazon Basin)	*P. vivax*	Concentrated hotspots; elimination feasible in low-burden areas
Western Pacific	Moderate–high in some islands	*P. falciparum*, *P. vivax*	Papua New Guinea and Solomon Islands highest outside Africa

Determinants influencing incidence include:

- Vector ecology: Abundance and efficiency of local *Anopheles* species.
- Parasite species mix: *P. falciparum* is associated with higher incidence and severity than *P. vivax*.
- Climate: Temperature, rainfall, and humidity patterns influence mosquito breeding and parasite development (Ryan *et al.* 2020).
- Socioeconomic factors: Poverty, inadequate housing, and limited access to preventive measures increase vulnerability.
- Health system capacity: Availability of diagnostics, effective treatment, and vector control directly impacts incidence trends.

Recent shifts and challenges:

- Stalled decline: After significant reductions from 2000–2015, incidence has plateaued or risen in some regions.
- System shocks: Disruptions such as the COVID-19 pandemic reduced prevention and treatment coverage (WHO, 2024).
- Resistance threats: Insecticide resistance in vectors and antimalarial resistance in parasites threaten current control tools (Hemingway *et al.* 2016).

1.1.2 World Malaria Report: Core Insights and Implications

The *World Malaria Report* provides an annual synthesis of malaria epidemiology, intervention coverage, and progress toward elimination. The 2024 edition highlights a rise in case numbers alongside relatively stable mortality rates, reflecting both the resilience of control programmes and the persistent challenge that hinder further gains (WHO, 2024).

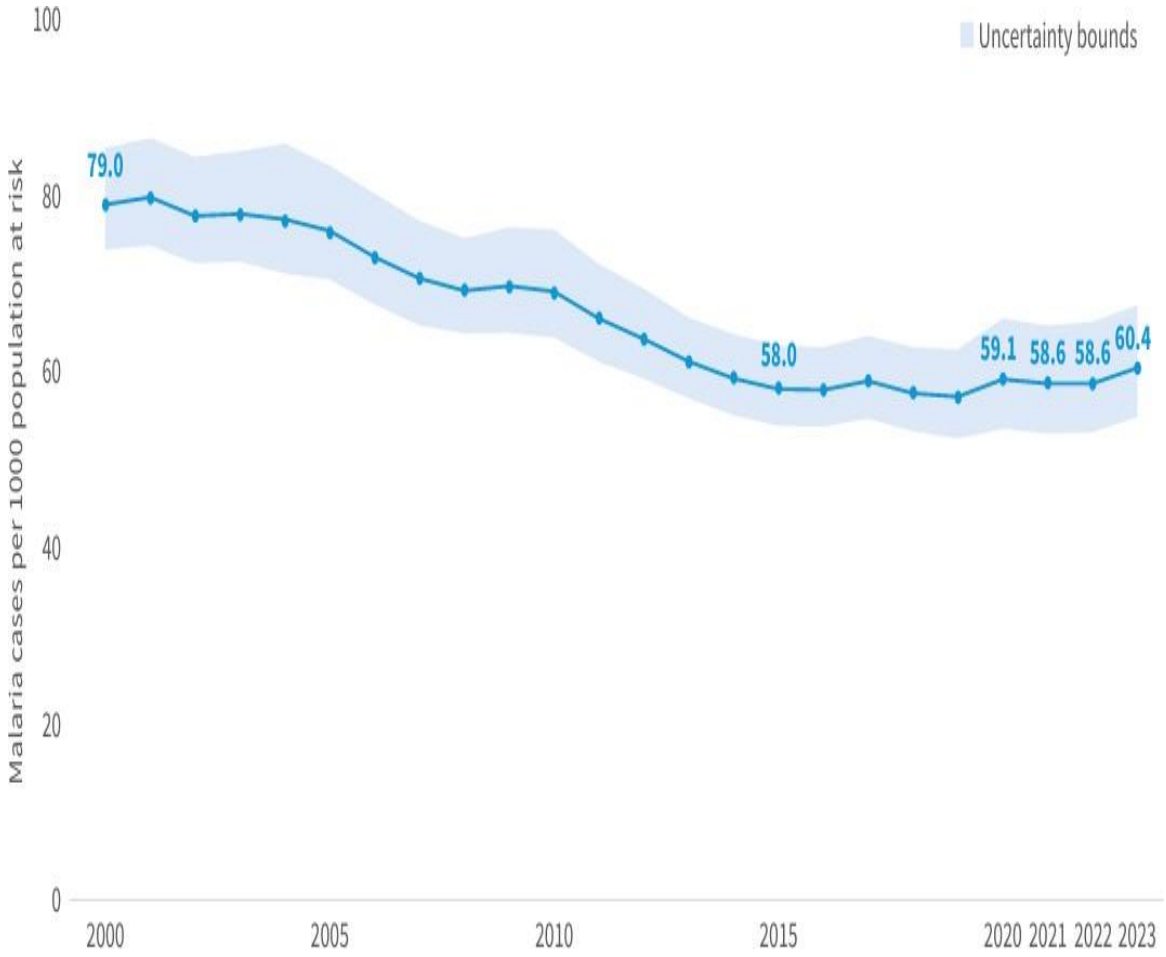

Figure 1.1: Global estimated malaria cases, 2000–2023

Global trends in malaria case incidence (cases per 1,000 populations at risk)

Source: Adapted from the World Malaria Report 2024 (WHO, 2024).

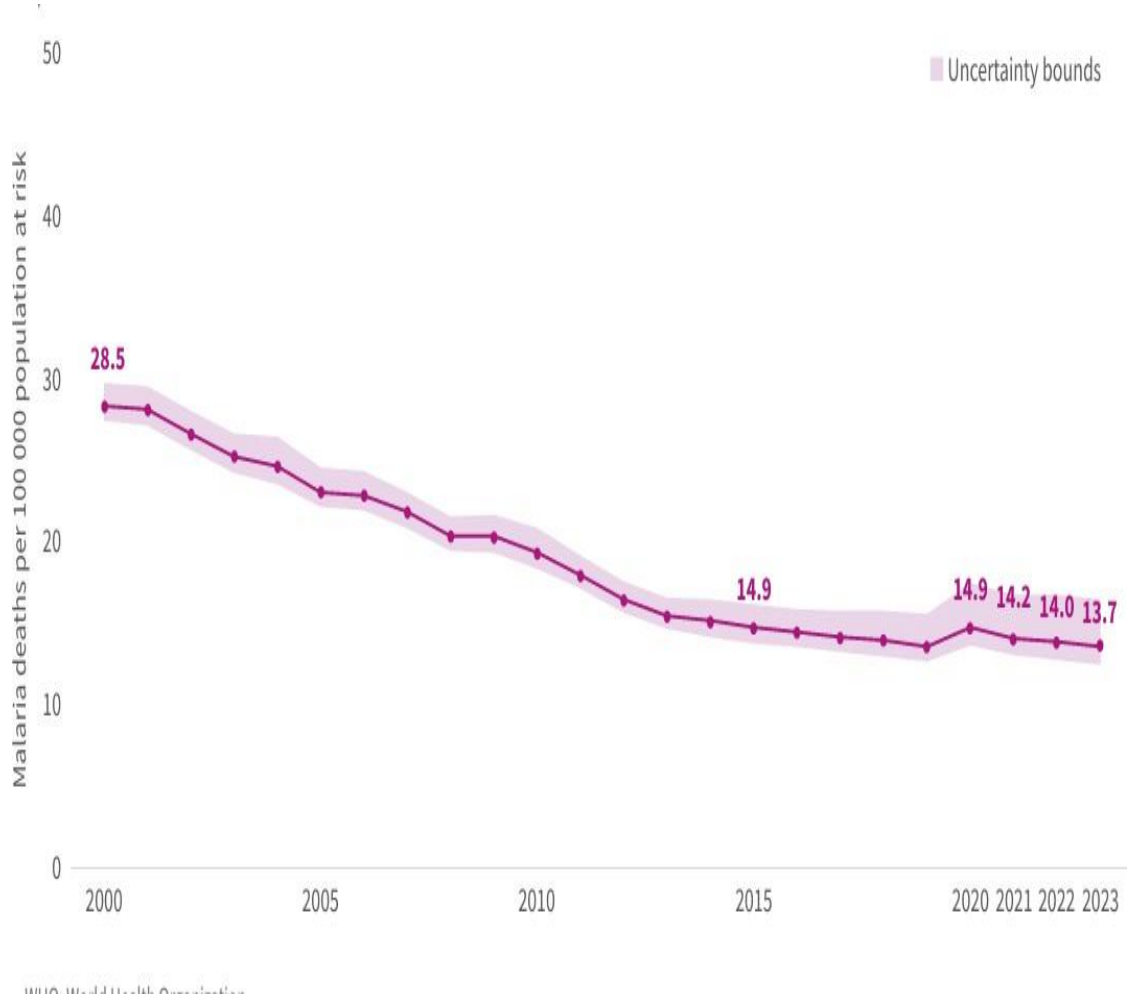

Figure 1.2: Global estimated malaria deaths, 2000–2023

Global trends in malaria mortality rate (deaths per 100,000 population at risk)

Source: Adapted from the World Malaria Report 2024 (WHO, 2024).

Burden and vulnerable groups:

- Global burden: Case numbers increased compared to the previous year, though mortality remained stable.
- Disproportionate impact: The African Region accounts for the vast majority of malaria deaths.
- Children under five: This group remains the most vulnerable, representing the largest share of malaria-related deaths.

Progress toward elimination:

- Certification momentum: Over 40 countries and territories have achieved malaria-free status, with new certifications in 2023–2024.
- Low-burden transitions: Many endemic countries now report fewer than 10,000 annual cases, signaling feasible elimination pathways.

Key interventions and innovations:

Intervention	Primary Purpose	Notes for Programmes
Insecticide-treated nets (ITNs)	Personal vector protection	New dual-ingredient nets counter pyrethroid resistance
Indoor residual spraying (IRS)	Indoor vector control	Requires insecticide rotation to manage resistance
Rapid diagnostic tests (RDTs)	Case confirmation at point of care	Reliable supply chains are essential
ACTs (e.g., Artemether/Lumefantrine)	Highly effective treatment	Adherence and food-effect counselling improve outcomes
Malaria vaccines (RTS,S/AS01; R21/Matrix-M)	Paediatric prevention	Best integrated with seasonal chemoprevention and ITNs

Persistent challenges:

- Insecticide resistance: Reduces ITN/IRS effectiveness without next-generation products.
- Drug resistance: Partial artemisinin resistance and partner drug resistance require vigilant surveillance (Conrad & Rosenthal, 2019).
- Climate change: Expands or shifts transmission zones.
- Access inequities: Remote and marginalised populations remain underserved.

Addressing these challenges requires targeted resource allocation, innovation in vector control, safeguarding ACT efficacy, and scaling up vaccine deployment where appropriate.

1.1.3 The Plasmodium Parasite: Species, Transmission, and Life Cycle

Malaria is caused by protozoan parasites of the genus *Plasmodium*, belonging to the phylum Apicomplexa and family Plasmodiidae. These are obligate intracellular parasites, meaning they must invade and replicate within host cells to complete their life cycle (GeeksforGeeks, 2025). Over 200 *Plasmodium* species have been identified, but only five are of major medical importance in humans (Biology Insights, 2025).

Medically Important Species

1. Plasmodium falciparum: The most virulent species, responsible for the majority of severe malaria cases and deaths. It multiplies rapidly in the blood and can cause cerebral malaria, severe anaemia, and multi-organ failure if untreated.
2. Plasmodium vivax: The most geographically widespread species outside Africa. It forms dormant liver stages (*hypnozoites*) that can reactivate months or years later, causing relapses.
3. Plasmodium ovale: Less common, found mainly in West Africa and some Pacific islands. Like *P. vivax*, it can form hypnozoites.

4. Plasmodium malariae: Causes a chronic, low-grade infection that can persist for years and is associated with nephrotic syndrome.
5. Plasmodium knowlesi: A zoonotic species primarily infecting macaques but capable of causing severe malaria in humans, particularly in Southeast Asia. It has a rapid 24-hour replication cycle (Citizens Hospitals, 2024).

Transmission

The primary mode of transmission is through the bite of an infected female *Anopheles* mosquito, which injects sporozoites into the human bloodstream during a blood meal. Less common routes include:

- Congenital transmission (mother to fetus)
- Blood transfusion or organ transplantation from infected donors
- Needle sharing among intravenous drug users (CDC, 2024)

Life Cycle Overview

The *Plasmodium* life cycle alternates between two hosts:

- Human host; site of asexual reproduction
- Mosquito vector; site of sexual reproduction

A. Mosquito Stage (Sporogony - Sexual Phase)

1. Mosquito ingests male and female gametocytes during a blood meal.
2. Fertilisation occurs in the mosquito gut, forming a zygote.
3. The zygote develops into an ookinete, penetrates the gut wall, and forms an oocyst.
4. Thousands of sporozoites develop within the oocyst and migrate to the salivary glands.

B. Human Liver Stage (Exo-erythrocytic Schizogony)

1. Sporozoites enter the bloodstream and invade hepatocytes.

2. They mature into schizonts, which rupture to release merozoites.
3. In *P. vivax* and *P. ovale*, some sporozoites become dormant hypnozoites.

C. Human Blood Stage (Erythrocytic Schizogony)

1. Merozoites invade red blood cells (RBCs) and develop through ring, trophozoite, and schizont stages.
2. Schizonts rupture, releasing merozoites and causing cyclical fevers.
3. Some merozoites differentiate into gametocytes, which are taken up by mosquitoes to continue the cycle (Biology Insights, 2025).

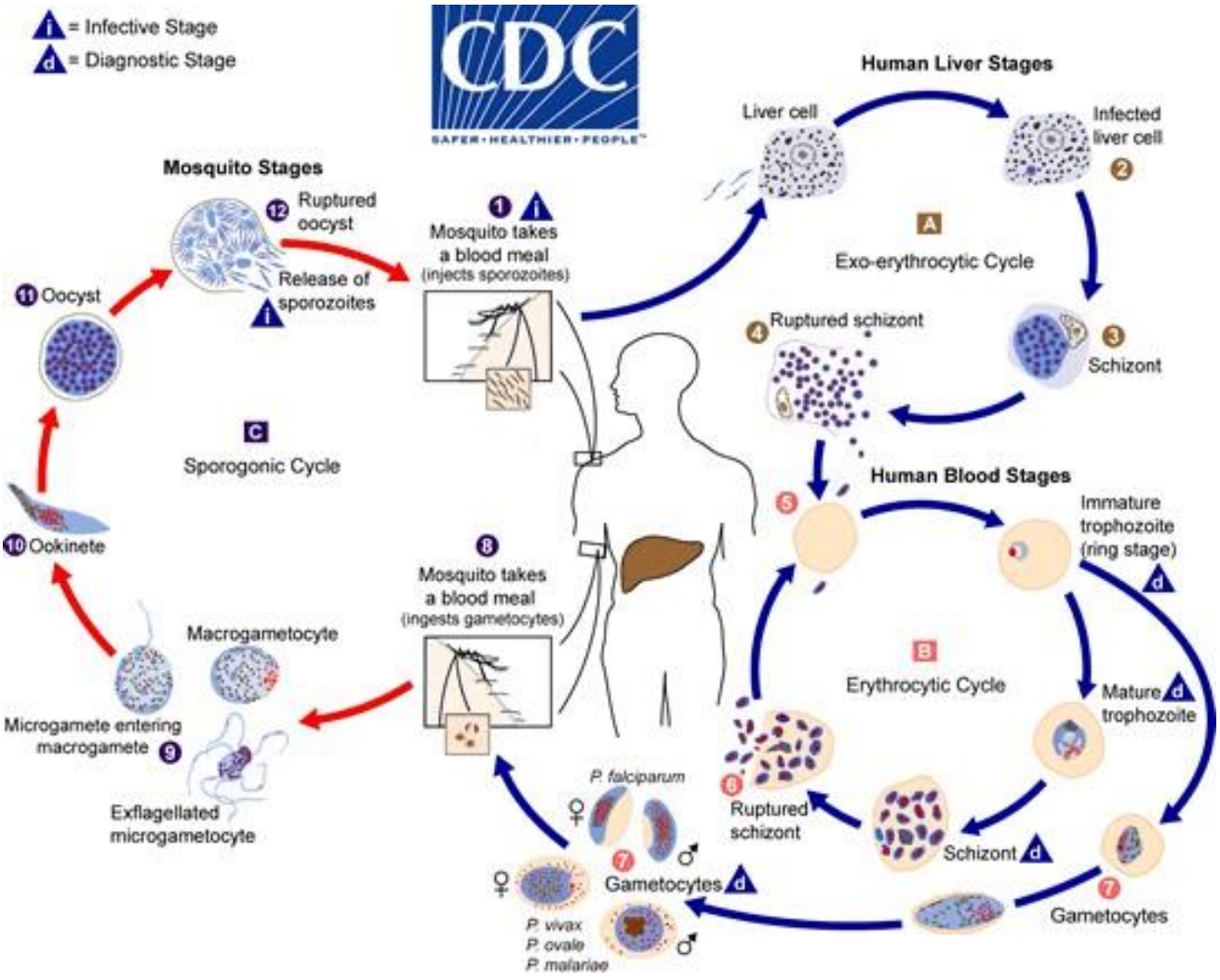

Figure 1.3: Life cycle of Plasmodium spp., showing mosquito and human stages (Adapted from CDC, DPDx Malaria).

Pathogenicity and Clinical Relevance

- RBC destruction → anaemia
- Parasite toxins → fever, chills, inflammation
- Severe *P. falciparum* → cerebral malaria, respiratory distress, multi-organ failure (WHO, 2024)

1.1.4 Classification of Malaria Infections

Malaria can be classified by causative species or clinical presentation (Stanford Health Care, 2024; Citizens Hospitals, 2024).

A. By Species

Species	Severity & Features	Geographic Distribution
P. falciparum	Most dangerous; rapid multiplication; severe anaemia, cerebral malaria, multi-organ failure	Predominantly sub-Saharan Africa; also Asia, South America
P. vivax	Less fatal but debilitating; relapses due to hypnozoites	Asia, Latin America, parts of Africa
P. ovale	Rare, milder; relapse potential	West Africa, some Pacific islands
P. malariae	Chronic, low-grade; can persist for years; linked to nephrotic syndrome	Patchy worldwide
P. knowlesi	Zoonotic; rapid 24-hour cycle; can cause severe illness	Southeast Asia

B. By Clinical Presentation

1. Uncomplicated Malaria – Fever, chills, headache, malaise; no severe organ dysfunction; caused by any species.
2. Severe (Complicated) Malaria – Most often *P. falciparum*; features include cerebral malaria, severe anaemia, acute kidney injury, respiratory distress, hypoglycaemia, or shock.
3. Relapsing Malaria – Caused by *P. vivax* and *P. ovale* due to reactivation of hypnozoites.
4. Chronic Malaria – Most often *P. malariae*; persistent low-level parasitaemia, sometimes leading to kidney complications.

The following flowchart aids in the clinical differentiation of malaria types based on the infecting species:

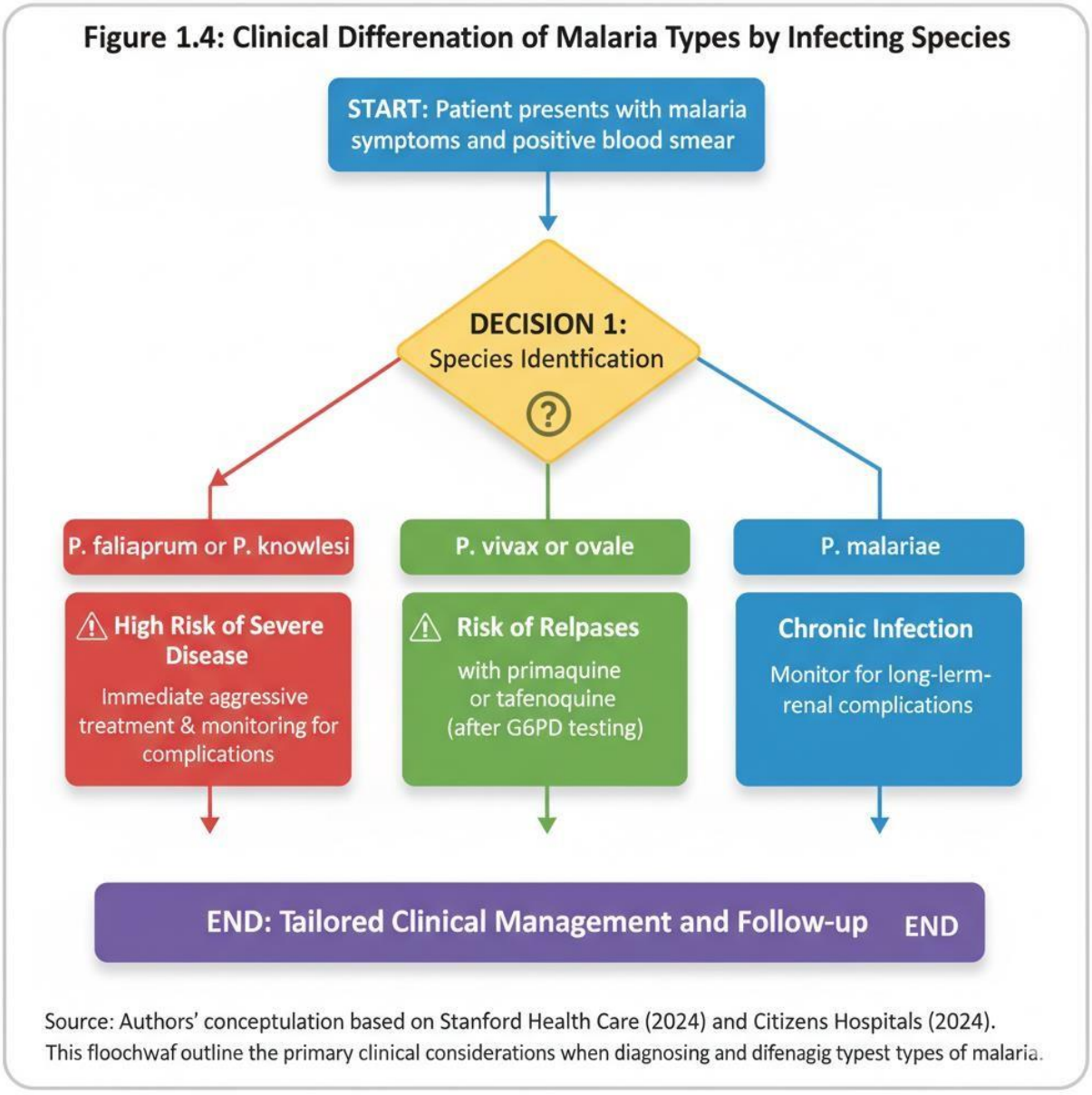

Figure 1.4: Clinical Differentiation of Malaria Types by Infecting Species

This flowchart outlines the primary clinical considerations when diagnosing and managing different types of malaria.

Source: Authors' conceptualization based on Stanford Health Care (2024) and Citizens Hospitals (2024).

1.1.5 Antimalarial Drugs

Antimalarial drugs are a diverse group of agents used for the treatment and prevention of malaria. They differ in chemical class, mechanism of action, pharmacokinetics, and target stage of the *Plasmodium* life cycle (Ashley *et al.* 2018). Understanding these differences is essential for effective case management, preventing resistance, and ensuring patient safety.

Classification by Chemical Class and Mechanism:

Drug	Class	MoA	Target Stage(s)	Side Effects	Adult Dose	Spectrum of Activity	Precautions
Chloroquine	4-aminoquinoline	Inhibits heme polymerase → toxic heme buildup	Asexual erythrocytic	Pruritus, GI upset, visual disturbance	600 mg base, then 300 mg base at 6, 24, 48 h	*P. vivax*, *P. ovale*, *P. malariae*, sensitive *P. falciparum*	Avoid in psoriasis, retinal disease; resistance common
Quinine	Quinoline methanol	Disrupts hemoglobin digestion	Asexual erythrocytic	Tinnitus, hypoglycemia, cinchonism	600 mg salt q8h × 7 days	All species (blood stages)	Caution in G6PD deficiency, tinnitus

Mefloquine	Quinoline methanol	Alters parasite membrane function	Asexual erythrocytic	Neuropsychiatric effects	750 mg, then 500 mg after 6–12 h	*P. falciparum*, *P. vivax*	Avoid in psychiatric disorders
Lumefantrine	Arylamino-alcohol	Inhibits heme detoxification	Asexual erythrocytic	GI upset, QT prolongation	120 mg with 20 mg artemether bid × 3 days	*P. falciparum*	Avoid with QT-prolonging drugs
Primaquine	8-aminoquinoline	Disrupts parasite mitochondria; kills hypnozoites	Liver hypnozoites (*P. vivax*, *P. ovale*) & gamet	Hemolysis in G6PD deficiency	15 mg base daily × 14 days	Radical cure of *P. vivax*, *P. ovale*	Avoid in pregnancy, G6PD deficiency

			oc ytes				
Artemisinin derivatives (e.g., artemether, artesunate)	Sesquiterpene lactone endoperoxide	Generates free radicals → protein damage	Asexual erythrocytic & early gametocytes	Dizziness, nausea	Artesunate: 2.4 mg/kg IV at 0, 12, 24 h, then daily	All species	Use in combination to prevent resistance

Note: The choice of drug depends on the *Plasmodium* species, drug resistance patterns, patient factors (e.g., pregnancy, comorbidities), and severity of disease (WHO, 2023).

The following diagram illustrates the primary sites of action of these key drugs within the *Plasmodium* life cycle.

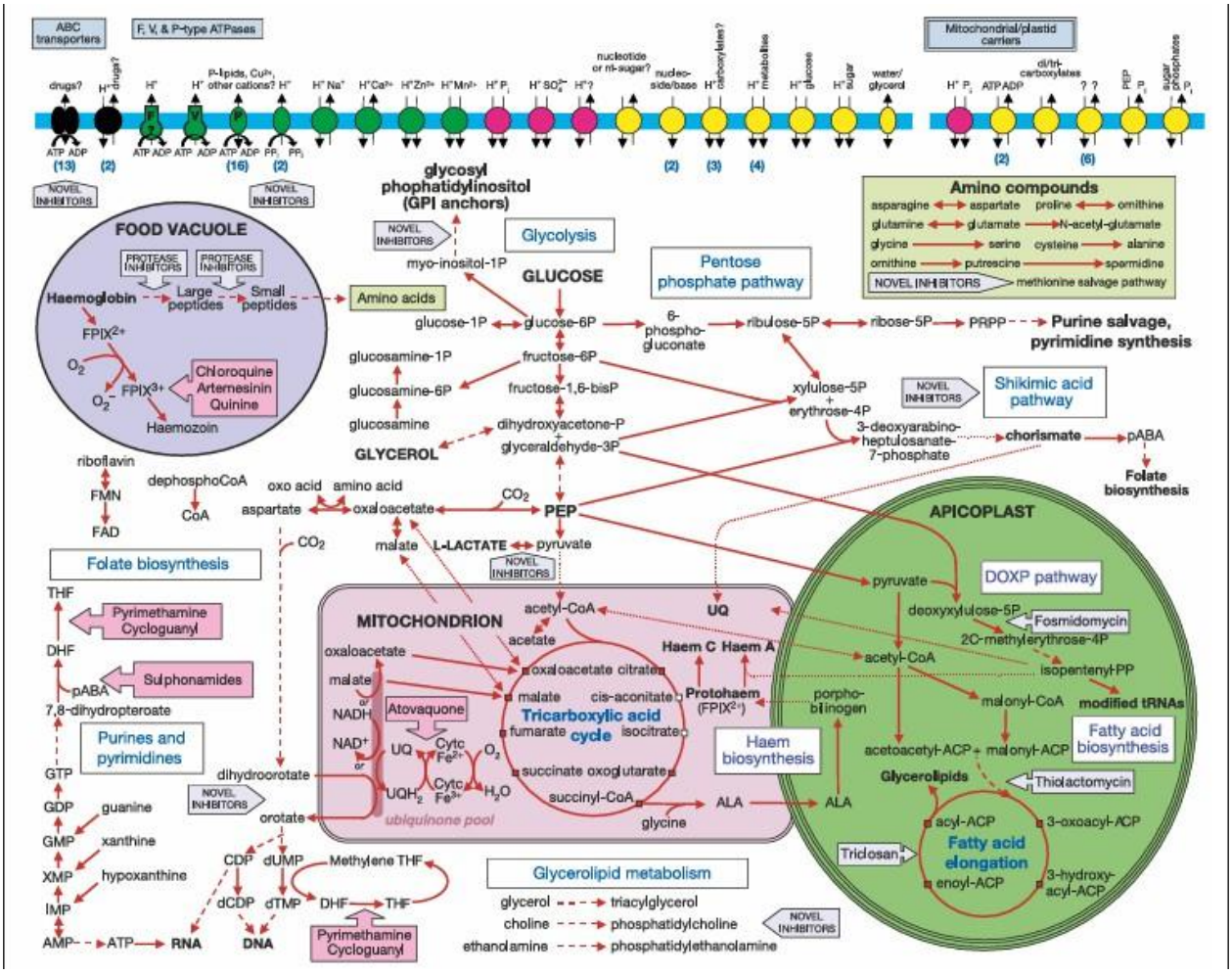

Figure 1.5: Sites of Action of Major Antimalarial Drug Classes on the *Plasmodium* Life Cycle

This diagram shows where different classes of antimalarial drugs interrupt the parasite's development in the human and mosquito hosts.

Source: Genome sequence of the human malaria parasite Plasmodium falciparum (Nature, 2002)

1.1.6 Artemisinin-Based Combination Therapies (ACTs)

Definition and Rationale

ACTs combine a fast-acting artemisinin derivative with a longer-acting partner drug. The artemisinin component rapidly reduces parasite biomass, while the partner drug clears residual parasites and prevents recrudescence (WHO, 2018).

Historical Context

Artemisinin was isolated in the 1970s from *Artemisia annua* by Tu Youyou, whose work earned the 2015 Nobel Prize in Physiology or Medicine (Tu, 2016). Early monotherapy was effective but associated with high relapse rates due to the short half-life of artemisinin. Combining it with a long-acting partner drug addressed this limitation and reduced the risk of resistance.

Pharmacological Features

- Artemisinin derivatives (e.g., artemether, artesunate, DHA) contain an endoperoxide bridge essential for activity.
- They achieve a ~10,000-fold reduction in parasite biomass per asexual cycle (White *et al.* 2014).
- Partner drugs (e.g., lumefantrine, amodiaquine, piperaquine) have longer half-lives.

Advantages

- Rapid symptom resolution
- Reduced risk of resistance compared to monotherapy

Resistance Concerns

- Artemisinin resistance: Delayed parasite clearance, linked to *kelch13* mutations.
- Partner drug resistance: Can occur independently, threatening ACT efficacy (Conrad & Rosenthal, 2019).

Table 1.2: Examples of WHO-recommended ACTs

ACT Combination	**Artemisinin Component**	**Partner Drug**	**Notes**
Artemether-Lumefantrine (AL)	Artemether	Lumefantrine	Widely used; taken with fatty food to enhance absorption
Artesunate-Amodiaquine (AS-AQ)	Artesunate	Amodiaquine	Common in Africa; effective against chloroquine-resistant strains
Artesunate-Mefloquine (AS-MQ)	Artesunate	Mefloquine	Used in Southeast Asia; caution in psychiatric disorders
Dihydroartemisinin-Piperaquine (DHA-PPQ)	Dihydroartemisinin	Piperaquine	Long post-treatment prophylaxis; QT prolongation risk
Artesunate-Sulfadoxine-Pyrimethamine (AS-SP)	Artesunate	Sulfadoxine-Pyrimethamine	Used were SP resistance is low; contraindicated in sulfa allergy

1.1.7 Artemether/Lumefantrine (AL)

Overview

Artemether/Lumefantrine is a fixed-dose ACT for uncomplicated *P. falciparum* malaria. Artemether provides rapid parasite clearance; lumefantrine sustains suppression due to its longer half-life (Ezzet *et al.* 2000).

Pharmacological Rationale

- Artemether: Rapidly kills asexual blood-stage parasites and some early gametocytes via free radical damage.
- Lumefantrine: Inhibits heme detoxification, leading to toxic heme accumulation.

Composition

- 20 mg artemether + 120 mg lumefantrine per tablet.

Clinical Use

- First-line treatment for uncomplicated *P. falciparum* in many endemic countries.

Advantages

- Short, 3-day regimen improves adherence.
- Extensive safety record.

Limitations

- Food-dependent absorption of lumefantrine.
- QT prolongation risk.

Popular Brands in Nigeria

- Coartem® (Novartis)
- Lonart DS® (Greenlife)
- Amatem Forte® (Elbe Pharma)

Table 1.3: Artemether/Lumefantrine Key Features

Feature	Details
Artemisinin component	Artemether
Partner drug	Lumefantrine
Tablet strength	20 mg / 120 mg
Regimen	6 doses over 3 days
Food effect	Fatty meals ↑ lumefantrine absorption
Key risks	CYP3A4 interactions; QT prolongation

1.2 Drug Interaction

1.2.1 Overview

A drug interaction occurs when the pharmacological effect of a drug is altered by the presence of another substance. This substance may be another drug, a specific food or beverage, a herbal preparation, alcohol, an underlying disease state, a genetic variation, or even a laboratory testing condition (Holm *et al.* 2020).

From a clinical perspective, recognising and anticipating drug interactions is essential because they can:

- Reduce therapeutic efficacy; leading to treatment failure if drug concentrations fall below the minimum effective level.
- Increase toxicity risk; if drug concentrations rise above the safe therapeutic range.
- Produce beneficial effects; in some cases, interactions are intentional, such as combining drugs for synergistic action.

In malaria management, drug interactions are particularly important because many antimalarials, including Artemether/Lumefantrine (AL), have narrow therapeutic indices and are metabolised by the cytochrome P450 (CYP450) system, especially CYP3A4. This makes them susceptible to both enzyme induction (which can lower drug levels) and enzyme inhibition (which can raise drug levels), with potential consequences for efficacy and safety (Adedeji *et al.* 2018).

Types of Drug Interactions

Interaction Type	Description	Unique Features	Clinical Example
Drug–Drug (DDI)	Interaction between two or more drugs.	May be pharmacokinetic (affecting absorption, distribution, metabolism, or excretion) or pharmacodynamic (altering drug effects without changing concentrations).	AL with strong CYP3A4 inducers like rifampicin → reduced ACT efficacy.
Drug–Food	Food alters drug absorption, metabolism, or elimination.	Can enhance or reduce bioavailability.	Fatty meals significantly increase lumefantrine absorption in AL.
Drug–Herb	Herbal products modify drug activity.	Often via CYP enzyme or transporter modulation.	St John's Wort induces CYP3A4 → lowers AL plasma levels.
Drug–Gene	Genetic polymorphisms affect drug metabolism or transport.	Basis for pharmacogenomics and personalised dosing.	CYP2C8 variants alter amodiaquine metabolism.

Drug–Disease	Disease states change pharmacokinetics or pharmacodynamics.	Organ dysfunction can alter clearance or sensitivity.	Severe hepatic impairment slows lumefantrine clearance.
Drug–Lab Test	Drug interferes with laboratory assay accuracy.	May cause false results and misdiagnosis.	High-dose quinine can cause false insulin elevations in some assays.
Drug–Alcohol	Alcohol modifies drug effects or toxicity.	Acute vs. chronic effects differ; additive CNS depression possible.	Chronic alcohol + chloroquine → increased hepatotoxicity risk.

Clinical relevance to Artemether/Lumefantrine:

- CYP3A4 induction (e.g., by rifampicin, phenobarbital) can markedly reduce AL exposure, risking treatment failure.
- CYP3A4 inhibition (e.g., by ketoconazole, cimetidine) can raise lumefantrine levels, increasing the risk of QT interval prolongation.
- Food effect: Lumefantrine absorption is significantly enhanced by dietary fat; poor adherence to this recommendation can reduce efficacy (Ezzet *et al.* 2000).

1.2.2 Drug-Drug Interactions (DDIs)

Drug-drug interactions can occur at any stage of the ADME process; Absorption, Distribution, Metabolism, and Excretion and may be:

- Pharmacokinetic: altering the concentration of a drug in the body.
- Pharmacodynamic: altering the drug's effect without changing its concentration (Holm *et al.* 2020).

Because many antimalarials, including Artemether/Lumefantrine (AL), have narrow therapeutic indices and are metabolised by the CYP450 enzyme system, even modest changes in drug handling can have significant clinical consequences (White *et al.* 2014).

1. Absorption

Mechanism: One drug alters the gastrointestinal uptake of another by changing gastric pH, motility, or by binding in the gut lumen. Examples in antimalarials:

- Antacids containing magnesium/aluminium hydroxide reduce chloroquine absorption by forming insoluble complexes (Baird, 2017).
- Fatty meals enhance lumefantrine absorption in AL, a beneficial interaction that improves treatment efficacy (Ezzet *et al.* 2000).

2. Distribution

Mechanism: Displacement from plasma proteins or altered tissue binding changes the proportion of free (active) drug. Examples:

- Sulfonamides can displace highly protein-bound drugs such as warfarin, increasing bleeding risk.
- Quinine can displace digoxin from tissue binding sites, raising the risk of digoxin toxicity (Ashley *et al.* 2018).

3. Metabolism

Mechanism: Induction or inhibition of hepatic enzymes especially CYP450 isoforms — changes the rate of drug breakdown. Examples:

- Induction: Rifampicin induces CYP3A4, lowering artemether and lumefantrine levels, potentially causing ACT treatment failure (German *et al.* 2016).
- Inhibition: Cimetidine inhibits CYP3A4, which can increase lumefantrine exposure and raise the risk of QT prolongation.

4. Excretion

Mechanism: Competition for renal tubular secretion or changes in urinary pH can alter drug elimination. Examples:

- Quinine competes with digoxin for renal excretion, increasing digoxin levels.
- Probenecid co-administration can prolong the half-life of some antifolates by reducing renal clearance (Baird, 2017).

Clinical Relevance to Antimalarial Therapy

- Many antimalarials are CYP450 substrates, making them prone to clinically significant DDIs.

- QT prolongation risk can be amplified when AL is combined with other QT-prolonging drugs.
- Therapeutic failure can occur if enzyme induction reduces drug exposure below effective levels.

Table 1.4: Examples of clinically relevant DDIs with antimalarials

DDI Mechanism	**Example with Antimalarials**	**Clinical Consequence**
Absorption inhibition	Antacids + chloroquine	Reduced efficacy of chloroquine
Absorption enhancement	Fatty meal + AL	Improved lumefantrine bioavailability
Metabolism induction	Rifampicin + AL	Sub-therapeutic ACT levels → treatment failure
Metabolism inhibition	Cimetidine + lumefantrine	Higher lumefantrine levels → increased QT risk
Protein-binding displacement	Sulfonamides + warfarin	Increased anticoagulant effect, bleeding risk
Renal excretion competition	Quinine + digoxin	Higher digoxin levels, toxicity risk

1.2.3 The Cytochrome P450 (CYP450) Enzyme System

Cytochrome P450 Superfamily: Role in Drug Metabolism

The Cytochrome P450 (CYP450) enzymes are a large and diverse superfamily of heme-containing monooxygenases located predominantly in the smooth endoplasmic reticulum of hepatocytes, with smaller amounts present in the intestinal mucosa, lungs, kidneys, and brain (Guengerich, 2021). These enzymes catalyse Phase I metabolic reactions, including oxidation, reduction, and hydrolysis which introduce or expose functional groups on drug molecules. This transformation generally increases polarity, preparing compounds for Phase II conjugation and eventual elimination.

In pharmacology, CYP450 enzymes perform several critical functions:

- Drug clearance: Converting lipophilic drugs into more hydrophilic metabolites for excretion.
- Bioactivation: Transforming prodrugs into their active forms (e.g., codeine → morphine via CYP2D6).
- Detoxification: Metabolising potentially harmful compounds into less toxic forms.
- Mediation of drug–drug interactions: Acting as primary sites for enzyme inhibition and induction, which can significantly alter drug exposure.

Although CYP enzymes also metabolise endogenous molecules such as steroids, fatty acids, and vitamins, their clinical importance in pharmacotherapy lies in their broad substrate specificity for xenobiotics, including most antimalarial agents (Zanger & Schwab, 2013).

Major CYP450 Isoforms in Drug Metabolism

Over 50 human CYP isoforms have been identified, but a small subset is responsible for the majority of clinically significant drug metabolism.

Table 1.5: Major CYP450 isoforms and selected antimalarial substrates

Isoform	**Approx. % of Drug Metabolism**	**Key Non-Antimalarial Substrates**	**Example Antimalarial Substrates**
CYP3A4/5	~30-40%	Macrolide antibiotics, statins, benzodiazepines	Artemether, Lumefantrine, Piperaquine
CYP2D6	~20-25%	Beta-blockers, antidepressants, opioids	Quinine, Primaquine
CYP2C9	~10-15%	NSAIDs, warfarin, sulfonylureas	Proguanil (partial), some antifolates
CYP2C8	~5%	Thiazolidinediones (e.g., pioglitazone)	Amodiaquine
CYP2B6	<5%	Bupropion, efavirenz	Artemisinin (minor pathway)
CYP1A2	<5%	Caffeine, theophylline	Possibly quinine (partial metabolism)

1.2.4 Hepatic Enzyme Induction and Inhibition

Mechanism of Enzyme Induction and Its Clinical Consequences

Enzyme induction refers to an increase in the synthesis and/or catalytic activity of drug-metabolising enzymes, most often within the cytochrome P450 (CYP450) system, following exposure to certain xenobiotics (Henderson *et al.* 2019). This process is typically mediated by the activation of nuclear receptors such as:

- Pregnane X receptor (PXR)
- Constitutive androstane receptor (CAR)
- Aryl hydrocarbon receptor (AhR)

Once activated, these receptors bind to specific DNA response elements, upregulating the transcription of CYP genes and increasing the amount of enzyme available for metabolism.

Key pharmacological effects of induction:

- Accelerated metabolism of drugs that are CYP substrates.
- Reduced plasma concentrations and shortened half-life of affected drugs.
- Potential therapeutic failure if doses are not adjusted to compensate for increased clearance.

In malaria therapy, enzyme induction is clinically important because it can lower the exposure of Artemether/Lumefantrine (AL), potentially leading to sub-therapeutic levels and treatment failure.

Mechanism of Enzyme Inhibition and Its Clinical Impact

Enzyme inhibition occurs when a substance reduces the metabolic activity of a drug-metabolising enzyme, thereby slowing the breakdown of its substrates (U.S. Food and Drug Administration [FDA], 2020). This can be:

- Reversible inhibition:
    - *Competitive*: The inhibitor competes with the substrate for the same active site.
    - *Non-competitive*: The inhibitor binds to a different site, altering enzyme function.
    - Effect ceases when the inhibitor is removed.
- Irreversible inhibition:
    - The inhibitor permanently inactivates the enzyme, often via covalent binding.
    - Recovery requires synthesis of new enzyme molecules.

Key pharmacological effects of inhibition:

- Slower metabolism of CYP substrates.
- Higher plasma concentrations and prolonged drug effects.
- Increased risk of dose-related toxicity.

In the context of AL, inhibition of CYP3A4 can raise lumefantrine levels, which may increase the risk of QT interval prolongation and associated cardiac arrhythmias.

Examples of Known Inducers and Inhibitors:

Category	Examples	Target CYP Isoforms	Clinical Note
Inducers	Phenobarbital	CYP3A4, CYP2C9, CYP2C19	Enhances clearance of many antimalarials; used in this study as the positive induction control
	Rifampicin	CYP3A4, CYP2C9, CYP2C19	Markedly lowers ACT exposure; risk of malaria treatment failure
	Carbamazepine	CYP3A4	Reduces plasma levels of artemisinin derivatives
Inhibitors	Cimetidine	Multiple CYPs (CYP3A4, CYP2D6, CYP1A2)	Slows clearance of several antimalarials; used in this study as the positive inhibition control
	Ketoconazole	Potent CYP3A4 inhibitor	Raises lumefantrine levels; may increase QT prolongation risk
	Clarithromycin	CYP3A4 inhibitor	Can prolong QT interval when combined with lumefantrine or piperaquine

Relevance to the present study: In this experimental design, phenobarbital and cimetidine serve as benchmark controls for induction and inhibition, respectively. Comparing AL's effect on hepatic CYP450 content against these controls will help determine whether AL behaves as an inducer, inhibitor, or has no significant modulatory effect.

1.3 Method Descriptions

1.3.1 Modified Lowry Method

The Modified Lowry Method is a widely used colorimetric assay for determining the protein concentration in biological samples, including liver microsomes. It is an adaptation of the original method developed by Lowry *et al.* (1951), with modifications that improve sensitivity, minimize interference from certain chemicals, and enhance compatibility with microsomal preparations (Waterborg, 2009).

The method combines two sequential reactions:

1. The Biuret reaction; formation of a copper-peptide complex under alkaline conditions.
2. The Folin-Ciocalteu reaction; reduction of a phosphomolybdic-phosphotungstic acid complex by the copper-protein complex, producing a blue chromogen measurable by spectrophotometry.

Principle

- Under alkaline conditions, cupric ions (Cu^{2+}) bind to peptide bonds in proteins, forming a copper-protein complex.
- This complex reduces the Folin-Ciocalteu reagent, resulting in a blue colour.
- The intensity of the colour, measured at 750 nm (or 660 nm in some protocols), is directly proportional to the protein concentration in the sample (Lowry *et al.* 1951).

Reagents and Their Functions

Reagent	Composition	Function in the Assay
Alkaline sodium carbonate solution	2% Na_2CO_3 in 0.1 M NaOH	Provides the alkaline medium necessary for copper–protein complex formation
Copper sulfate solution	0.5% $CuSO_4 \cdot 5H_2O$ in distilled water	Supplies cupric ions (Cu^{2+}) that bind to peptide bonds in proteins
Sodium potassium tartrate solution	1% Na-K tartrate in distilled water	Stabilises cupric ions in solution and prevents precipitation
Alkaline copper reagent (Reagent C)	Freshly mixed alkaline sodium carbonate + copper sulfate + sodium potassium tartrate	Reacts with peptide bonds to form the copper-protein complex
Folin–Ciocalteu phenol reagent	Phosphomolybdic–phosphotungstic acid complex	Reduced by the copper-protein complex to form a blue chromogen
Bovine serum albumin (BSA) standard	Known concentration (e.g., 2 mg/mL) in buffer or saline	Used to prepare a standard curve for quantifying unknown protein concentrations
Distilled water	Purified water	Used for reagent preparation and dilution of samples/standards

Microsomal protein sample	Liver microsome suspension	The test sample whose protein content is to be determined

Relevance to This Study

In this experiment, the Modified Lowry Method is used to determine the total protein concentration in rat liver microsomal preparations. This value serves as the denominator when expressing CYP450 content (nmol/mg protein), ensuring that comparisons between treatment groups reflect true enzyme modulation rather than differences in microsome yield.

1.3.2 Omura-Sato CO-difference method

Quantifying Cytochrome P450 (CYP450) content in liver microsomes is a critical step in evaluating hepatic enzyme activity. In this study, the Omura and Sato (1964) spectrophotometric carbon monoxide (CO) difference method is employed. This technique is widely regarded as the gold standard for measuring total CYP450 content because of its specificity, sensitivity, and reproducibility (Omura & Sato, 1964; Guengerich, 2021).

Principle of the Omura and Sato Method

CYP450 enzymes contain a heme prosthetic group whose iron atom can exist in two oxidation states:

- Ferric (Fe^{3+})
- Ferrous (Fe^{2+})

In the reduced ferrous state, the heme iron has a high affinity for carbon monoxide (CO). When CO binds to the reduced heme iron, it forms a CO-CYP450 complex that exhibits a characteristic absorbance peak at 450 nm in the visible spectrum.

This spectral shift is unique to CYP450 and distinguishes it from other hemoproteins such as cytochrome b_5, which has a peak at 424 nm (Omura & Sato, 1964).

Reagents and Their Functions

Reagent	Composition / Source	Function in the Assay
Potassium phosphate buffer	0.1 M, pH 7.4	Maintains pH and ionic strength for enzyme stability during measurement
Microsomal protein sample	Liver microsome suspension	Source of CYP450 enzymes to be quantified
Sodium dithionite	Freshly prepared in buffer	Reduces CYP450 heme iron from Fe^{3+} to Fe^{2+} state
Carbon monoxide (CO) gas	High-purity CO cylinder	Binds to reduced CYP450 to form CO–CYP450 complex with characteristic 450 nm absorbance
Distilled water	Purified water	Used for reagent preparation and as spectrophotometer blank

Relevance to This Study

In this experiment, the Omura & Sato method provides the absolute CYP450 content in nmol/mL for each microsomal preparation. When combined with the protein concentration obtained from the Modified Lowry Method (Section 1.3.1), the results can be expressed as nmol CYP450 per mg protein. This normalisation allows for valid comparisons between treatment groups, ensuring that observed differences reflect true enzyme modulation rather than variations in microsome yield.

1.3.3 Linking 1.3.1 and 1.3.2 in the Project Rationale

In this study, the Modified Lowry Method and the Omura & Sato CO-difference method are applied as complementary analytical techniques to generate a complete biochemical profile of hepatic microsomal preparations from each experimental group. Together, they ensure that the quantification of cytochrome P450 (CYP450) enzymes is both accurate and biologically meaningful.

Step 1 - Total Protein Quantification (Modified Lowry Method): This assay determines the total microsomal protein concentration (mg/mL) for each sample. Since CYP450 content is expressed relative to protein mass (nmol CYP450 per mg protein), this measurement serves as the denominator in the final calculations. Without this normalisation, differences in CYP450 values between groups could simply reflect variations in microsome yield or sample preparation efficiency, rather than true enzyme induction or inhibition (Lowry *et al.* 1951; Waterborg, 2009).

Step 2 - CYP450 Content Determination (Omura & Sato Method): This spectrophotometric assay measures the absolute amount of CYP450 hemoprotein in each microsomal sample (nmol/mL) by exploiting the unique absorbance peak of the CO-CYP450 complex at 450 nm (Omura & Sato, 1964). When paired with the protein concentration from Step 1, the data can be

expressed as nmol CYP450 per mg protein, enabling valid comparisons of enzyme levels across treatment groups.

Why Both Steps Are Essential in This Experimental Design:

- Phenobarbital group (positive induction control): Expected to show a significant increase in CYP450 content per mg protein compared to the olive oil negative control, confirming the assay's ability to detect enzyme induction.
- Cimetidine group (positive inhibition control): Expected to show a significant decrease in CYP450 content per mg protein compared to the negative control, confirming the assay's ability to detect enzyme inhibition.
- Artemether/Lumefantrine group (test drug): Any deviation from control values can be confidently attributed to AL's specific effect on CYP450 expression or activity, rather than differences in microsomal yield or protein concentration.

By integrating these two methods, the study ensures that the observed changes in CYP450 levels are quantitatively robust and biologically interpretable, providing a reliable basis for assessing whether Artemether/Lumefantrine acts as a CYP450 inducer, inhibitor, or has no significant modulatory effect.

1.4 Background of the Study

Malaria control strategies in endemic regions rely heavily on artemisinin-based combination therapies (ACTs) as the cornerstone of effective case management. Among these, Artemether/Lumefantrine (AL) is one of the most widely deployed regimens for the treatment of uncomplicated *Plasmodium falciparum* malaria (World Health Organization [WHO], 2023). AL combines a fast-acting artemisinin derivative (artemether) with a longer-acting partner drug (lumefantrine), achieving rapid parasite clearance and sustained suppression of residual parasites.

This dual-component approach not only improves cure rates but also reduces the risk of recrudescence and transmission (White *et al.* 2014).

Both artemether and lumefantrine undergo hepatic biotransformation, placing the cytochrome P450 (CYP450) enzyme system, particularly CYP3A4 at the center of their pharmacokinetics, efficacy, and safety profile (German *et al.* 2016). Artemether is extensively metabolised by CYP3A4 to dihydroartemisinin (DHA), which is subsequently cleared mainly via conjugation pathways. Lumefantrine is also a CYP3A4 substrate, with a relatively long terminal half-life and a recognised potential for QT interval prolongation at high plasma concentrations (Ezzet *et al.* 2000).

Importantly, artemisinin-class drugs have been reported to modulate CYP expression through activation of nuclear receptors such as the pregnane X receptor (PXR) and constitutive androstane receptor (CAR) (Li *et al.* 2014). This raises two clinically relevant questions:

1. Is AL a victim of CYP modulation by co-administered drugs, leading to altered exposure and efficacy?
2. Does AL itself modulate CYP450 enzymes - either inducing or inhibiting them, thereby affecting its own metabolism or that of other co-administered drugs?

These questions are particularly important in real-world polypharmacy scenarios common in malaria-endemic settings, where patients may also be receiving treatment for tuberculosis, HIV/AIDS, epilepsy, or other chronic conditions. Many of these co-medications are potent CYP inducers or inhibitors, increasing the risk of clinically significant drug-drug interactions (Holm *et al.* 2020).

Despite the widespread of AL use, controlled in vivo evidence on its direct effects on hepatic CYP450 content remains limited. Establishing whether AL induces, inhibits, or has no effect on

CYP450 enzymes is not merely an academic exercise - it has direct implications for dose adequacy, treatment durability, and safety monitoring, particularly with respect to cardiac risk from QT prolongation if lumefantrine exposure is increased.

In this study, a rat model is used to quantify total hepatic CYP450 content following AL administration. Three comparator groups are included:

- Phenobarbital - a prototypical CYP inducer (positive induction control)
- Cimetidine - a broad CYP inhibitor (positive inhibition control)
- Olive oil - vehicle negative control

By normalising CYP450 content to microsomal protein (nmol/mg protein), the study design ensures that observed differences reflect true enzyme modulation rather than variability in microsome yield or protein concentration. This approach provides a robust framework for determining AL's potential to alter hepatic CYP450 expression and activity.

1.5 Statement of the Problem

In malaria-endemic regions, patients treated with Artemether/Lumefantrine (AL) are often concurrently receiving other medications for co-existing conditions such as tuberculosis, HIV/AIDS, epilepsy, or chronic non-communicable diseases. Many of these co-medications are known modulators of the cytochrome P450 (CYP450) enzyme system either as inducers or inhibitors, and thus have the potential to alter AL's pharmacokinetics (Holm *et al.* 2020; German *et al.* 2016).

While AL is a first-line ACT recommended by the World Health Organization for uncomplicated *Plasmodium falciparum* malaria, there is limited in vivo evidence on whether AL itself acts as a CYP450 inducer or inhibitor. This knowledge gap is clinically significant because:

- If AL induces CYP450 enzymes, it could accelerate the metabolism of co-administered drugs, leading to sub-therapeutic concentrations and treatment failure.
- If AL inhibits CYP450 enzymes, it could slow the metabolism of co-administered drugs, increasing the risk of drug accumulation, toxicity, and in the case of lumefantrine, QT interval prolongation.

Current data are sparse, fragmented, and often extrapolated from studies on other artemisinin derivatives or ACT combinations rather than AL specifically. Moreover, few studies have employed rigorous in vivo models with validated positive and negative controls to confirm CYP450 modulation. Without such evidence, clinicians cannot reliably anticipate pharmacokinetic shifts, and guideline developers cannot make confident recommendations for co-medication use.

Therefore, directly testing AL for its potential to induce or inhibit hepatic CYP450 enzymes in a controlled experimental model, benchmarked against a known inducer (phenobarbital) and a known inhibitor (cimetidine) is a necessary step to de-risk its clinical use in polypharmacy scenarios and to inform evidence-based prescribing.

1.6 Justification of the Study

Public health impact: Malaria continues to impose a substantial health and economic burden in endemic regions, particularly in sub-Saharan Africa. Sustaining the effectiveness and safety of artemisinin-based combination therapies (ACTs) is critical to preserving the gains achieved in malaria control over the past two decades (WHO, 2023). As one of the most widely used ACTs, Artemether/Lumefantrine (AL) plays a pivotal role in reducing morbidity and mortality from *Plasmodium falciparum* malaria. Optimizing its use through a clear understanding of its interaction potential is therefore a public health priority.

High interaction potential: Both artemether and lumefantrine are primarily metabolised by CYP3A4, a key enzyme in the metabolism of many essential drugs used in the treatment of tuberculosis, HIV/AIDS, and epilepsy (German *et al.* 2016; Holm *et al.* 2020). This metabolic overlap increases the likelihood of clinically significant drug-drug interactions in real-world polypharmacy scenarios. Determining whether AL itself induces or inhibits CYP450 enzymes is essential for anticipating and managing such interactions.

Mechanistic clarity: The inclusion of phenobarbital (a well-established CYP inducer) and cimetidine (a broad-spectrum CYP inhibitor) as comparator groups provides biological "anchors" for interpreting results. These controls allow for unambiguous classification of AL's effects on CYP450 activity relative to validated extremes, strengthening the reliability of the findings (Henderson *et al.* 2019).

Clinical and regulatory relevance: The results of this study have the potential to inform prescribing guidelines, pharmacovigilance priorities (e.g., QT interval monitoring), and local formulary decisions. Evidence-based recommendations on co-medication use with AL could reduce the risk of therapeutic failure or adverse drug reactions, thereby improving patient outcomes.

Feasibility and rigour: The study employs two complementary, gold-standard analytical techniques, the Omura-Sato CO-difference method for quantifying total CYP450 content and the Modified Lowry Method for determining microsomal protein concentration. Normalising CYP450 content to protein mass ensures robust, comparable metrics across experimental groups, enabling accurate assessment of enzyme modulation

1.7 Research Hypothesis

Null hypothesis (H_0-AL): Administration of Artemether/Lumefantrine does not significantly alter hepatic CYP450 content (expressed as nmol CYP450 per mg microsomal protein) compared with the olive oil negative control.

Alternative hypothesis (H_1-AL): Administration of Artemether/Lumefantrine significantly alters hepatic CYP450 content compared with the olive oil control, in a manner consistent with either enzyme induction (increase) or enzyme inhibition (decrease).

Method validation expectations (assay performance checks)

To confirm the validity of the experimental approach:

- Phenobarbital (positive induction control) is expected to produce a significant increase in CYP450 content per mg protein relative to olive oil, demonstrating the assay's ability to detect enzyme induction.
- Cimetidine (positive inhibition control) is expected to produce a significant decrease in CYP450 content per mg protein relative to olive oil, demonstrating the assay's ability to detect enzyme inhibition.

Failure to observe these expected control effects would call into question the reliability of the assay results (Henderson *et al.* 2019).

Operational definitions for interpretation

- Induction: A statistically significant increase in CYP450 content (nmol/mg protein) compared with olive oil, directionally consistent with the phenobarbital control.
- Inhibition: A statistically significant decrease in CYP450 content compared with olive oil, directionally consistent with the cimetidine control.

- No modulation: No statistically significant difference in CYP450 content compared with olive oil.

1.8 Aim of the Study

The primary aim of this study is to determine whether Artemether/Lumefantrine (AL) acts as an inducer or inhibitor of hepatic cytochrome P450 (CYP450) enzymes in rats. This will be achieved by quantifying total hepatic CYP450 content, normalised to microsomal protein concentration, and benchmarking the observed effects against three comparator groups:

- Phenobarbital; a well-established CYP inducer (positive induction control)
- Cimetidine; a broad-spectrum CYP inhibitor (positive inhibition control)
- Olive oil; vehicle negative control

1.9 Specific Objectives

To achieve the stated aim, this study will pursue the following specific objectives:

1. To administer Artemether/Lumefantrine (AL), phenobarbital (induction control), cimetidine (inhibition control), and olive oil (negative control) to experimental rats and monitor for mortality and signs of acute toxicity throughout the dosing period.
2. To record and compare the body weights of all animals before and after the drug administration period as an indicator of overall health and systemic drug tolerability.
3. To isolate and prepare liver microsomal fractions from all experimental groups and determine the total microsomal protein concentration for each sample using the Modified Lowry colorimetric assay.
4. To quantify the absolute concentration of total hepatic CYP450 in each microsomal preparation using the established Omura and Sato CO-difference spectrophotometric method.

5. To calculate the normalized CYP450 content (nmol per mg of microsomal protein) for each animal to enable a valid comparison across all treatment groups.
6. To evaluate the inhibitory potential of AL by statistically comparing the normalized CYP450 content in the AL group to that of the cimetidine and olive oil control groups.
7. To evaluate the inductive potential of AL by statistically comparing the normalized CYP450 content in the AL group to that of the phenobarbital and olive oil control groups.
8. To interpret the overall CYP450 modulatory profile of AL (as inducer, inhibitor, or neutral) based on the statistical significance and direction of the observed changes relative to the positive and negative controls.

CHAPTER TWO

2.1 Materials

2.1.1 Test drug, chemicals, and other reagent

All chemicals and reagents used in the study were of analytical grade and were sourced from reputable suppliers. The materials used included:

- Artemether/Lumefantrine tablets (80 mg/480 mg per tablet): Obtained from a registered pharmacy in Benin City, Edo State, Nigeria.
- Phenobarbital sodium**:** Used as the enzyme induction control, purchased from a registered pharmacy in Benin City, Edo State, Nigeria.
- Cimetidine hydrochloride: Used as the enzyme inhibition control, also obtained from a registered pharmacy in Benin City, Edo State, Nigeria.
- Olive oil: Pharmaceutical grade, used as the vehicle for drug administration.
- Bovine serum albumin (BSA**)**: Used for protein standard preparation in the Modified Lowry assay.
- Folin–Ciocalteu reagent: Commercial stock solution, diluted 1:1 with distilled water before use.
- Alkaline copper reagent components: Including sodium carbonate, sodium hydroxide, copper(II) sulfate pentahydrate, sodium potassium tartrate, and sodium dodecyl sulfate.
- Sodium cholate**,** Triton X-100**,** and glycerol**:** Used in the preparation of the microsomal dilution buffer.

- Sodium dithionite: Used for chemical reduction in the CYP450 assay.
- Carbon monoxide (CO) gas: High-purity grade, delivered via fine-bore tubing.
- Potassium phosphate buffer (100 mM, pH 7.4-7.7): Prepared by mixing monobasic and dibasic phosphate salts and adjusted with a calibrated pH meter.
- EDTA (1.0 mM)**:** Added to the buffer to chelate divalent metal ions.
- Sucrose (0.25 M) and Tris–HCl (10 mM, pH 7.4): Used in the preparation of liver homogenates and microsomal fractions.
- Distilled water: Used throughout for reagent preparation and sample dilution.

All weighing and solution preparation were performed using calibrated analytical balances and volumetric glassware.

The study also made use of:

- UV-Vis spectrophotometer: Capable of fixed-wavelength readings at 420 nm, 450 nm, and 490 nm.
- Microplate reader: Configured to read at the same wavelengths for parallel sample analysis.
- Refrigerated centrifuge: Used for microsomal isolation at specified rpm and durations.
- Mortar and pestle**,** ice bath**,** quartz cuvettes**,** and optically clear microplates: Used during sample preparation and analysis.

2.1.2 Experimental Animals

The study made use of twenty-four (24) healthy albino rats (*Rattus norvegicus*), of both sexes, obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. At the time of procurement, the animals weighed between 130 g and 180 g**.**

Upon arrival, the rats were housed in clean, well-ventilated polypropylene cages bedded with wood shavings, under standard laboratory conditions of 25 ± 5 °C temperature, and a 12-hour light/12-hour dark cycle**.** They were allowed free access to standard growers' mash and clean drinking water *ad libitum*.

The animals were acclimatised to the laboratory environment for 14 days prior to the commencement of dosing. During this period, they were observed daily for signs of ill health or abnormal behaviour.

2.2 Method

2.2.1 Experimental Design

The twenty-four rats used in the study were randomly assigned into four groups**,** each comprising six animals (four males and two females). The groups were designed to evaluate the potential inductive or inhibitory effect of artemether/lumefantrine **(**AL**)** on hepatic cytochrome P450 (CYP450) enzymes, using phenobarbital and cimetidine as reference controls for enzyme induction and inhibition, respectively. Olive oil served as the vehicle control.

The experimental groups were as follows:

- Group A (Vehicle Control): Received olive oil only.
- Group B (Inhibition Control): Received cimetidine.
- Group C (Induction Control): Received phenobarbital**.**
- Group D (Test Group): Received artemether/lumefantrine**.**

All treatments were administered orally once daily for 14 consecutive days, except for the test group, which received artemether/lumefantrine twice daily to reflect its clinical dosing regimen. The olive oil vehicle was used to dissolve all test and control drugs, and dosing volumes were calculated based on individual body weights recorded prior to treatment initiation.

Each rat was identified using a tail-marking scheme with non-toxic permanent markers. Colours and numbers were assigned to distinguish individual animals within each group, ensuring accurate tracking throughout the study.

On Day 15**,** all animals were sacrificed under chloroform anaesthesia, and liver tissues were harvested for microsomal preparation and subsequent biochemical analysis.

2.2.2 Experimental Procedure

The rats were weighed individually prior to the commencement of dosing, and the recorded weights were used to calculate the appropriate volume of drug or vehicle to be administered. All treatments were administered via oral gavage. The dosing volume was standardised at 2.5 mL/kg per administration.

Dosing was carried out once daily for 14 consecutive days in the control groups, while the test group received twice-daily dosing to reflect the clinical regimen of artemether/lumefantrine.

On Day 15, all animals were sacrificed under chloroform anaesthesia, and the livers were excised immediately for microsomal preparation. Approximately 2g of liver tissue was collected from each animal and homogenised in 2mL of ice-cold 0.25 M sucrose solution using a mortar and pestle. The homogenates were stored overnight at -80 **°C** in a Laboratory freezer prior to microsomal isolation and biochemical analysis.

2.2.3 Drug Administration and Dose Calculation

Each group's stock solution was prepared by dissolving the adult 60 kg human dose of the assigned drug in 150 mL olive oil . The dose administered to each rat was calculated based on its body weight and the dosing volume was kept consistent at 2.5 mL/kg per administration.

Rationale and Calculation of Stock Solution Volume (150 mL)

The 150 mL volume was derived from scaling the per-rat dose to the adult dose as follows:

- Step 1: From dosing guidelines, 1 tablet is the standard dose for a 60 kg adult.

- Step 2: The average experimental rat weight was taken as 0.2 kg (200 g).
- Step 3: The proportional tablet fraction for a 0.2 kg rat is:

$$\frac{0.2\ \text{kg}}{60\ \text{kg}} = 0.0033\ \text{tablet}$$

- Step 4: In preliminary dosing, 0.0033 tablet corresponded to 0.5 mL (average volume for drug administration in rat) of prepared solution for an average rat.
- Step 5: To find the total volume equivalent to 1 tablet:

$$0.0033\ \text{tablet} \equiv 0.5\ \text{mL}$$

$$1\ \text{tablet} \equiv \frac{0.5}{0.0033} \cong 150\ \text{mL}$$

Thus, dissolving the adult dose (1 tablet) in 150 mL olive oil ensures that 0.5 mL delivers the correct scaled dose for a 0.2 kg rat.

Rationale and Calculation of the Dose Factor (2.5 mL/kg)

The dose factor represents the volume of stock solution to be administered per kilogram of rat body weight.

- Step 1: For an average 0.2 kg rat, the daily dose volume was 0.5 mL.
- Step 2: To scale this to a per-kilogram basis:

$$\text{Dose factor} = \frac{0.5\ \text{mL}}{0.2\ \text{kg}} = 2.5\ \text{mL/kg}$$

- Step 3: This factor was then applied to each rat's actual body weight to calculate the precise daily volume:

$$\text{Volume (mL)} = \text{Body weight (kg)} \times 2.5$$

Group A – Vehicle Control (Olive Oil)

Animal ID	Sex	Weight at day 0 (g)	Weight at day 15 (g)	Volume (mL/day)
OIL-R1	M	156.4	185.7	0.39
OIL-R2	M	203.1	212.2	0.5
OIL-B1	F	153.1	201.1	0.38
OIL-B2	F	174.1	183.2	0.4
OIL-G1	M	214.5	233.7	0.53
OIL-G2	M	165.0	192.1	0.4

Group B – Inhibition Control (Cimetidine, 400 mg/tablet)

Animal ID	Sex	Weight at day 0 (g)	Weight at day 15 (g)	Volume (mL/day)	Cimetidine (mg/day)
CIM-R1	M	161.2	166.6	0.4	1.067
CIM-R2	M	236.5	Deceased	0.6	1.600
CIM-B1	F	209.7	183.3	0.5	1.333
CIM-B2	F	181.0	184.6	0.45	1.200
CIM-G1	M	202.2	220.4	0.43	1.147
CIM-G2	M	171.9	177.1	0.51	1.360

Group C – Induction Control (Phenobarbital, 30 mg/tablet)

Animal ID	Sex	Weight at day 0 (g)	Weight at day 15 (g)	Volume (mL/day)	Phenobarbital (mg/day)
PHB-R1	M	153.4	173.7	0.38	0.077
PHB-R2	M	146.3	167.3	0.36	0.072
PHB-B1	F	164.0	220.3	0.41	0.083
PHB-B2	F	145.5	166.7	0.37	0.074
PHB-G1	M	146.2	152.1	0.37	0.074
PHB-G2	M	158.6	180.6	0.40	0.080

Group D - Test Group (Artemether/Lumefantrine, 80 mg/480 mg per tablet)

Rat ID	Weight Before (g)	Weight at day 0 (g)	Weight at day 15 (g)	Lumefantrine (mg/day)	Volume (mL/day)
AL-R1	157.9	192.4	0.213	1.280	0.4
AL-R2	152.3	195.8	0.213	1.280	0.4
AL-B1	155.6	182.6	0.213	1.280	0.4
AL-B2	130.7	153.3	0.176	1.056	0.33
AL-G1	172.5	205.3	0.229	1.376	0.43
AL-G2	146.2	170.6	0.197	1.184	0.37

2.2.4 Sample Collection and Preparation of Liver Homogenates

On Day 15, all animals were sacrificed under chloroform anaesthesia. Following confirmation of deep anaesthesia, the abdominal cavity was opened and the entire liver was excised from each rat. Approximately 2 g of liver tissue was collected per animal and placed in 2 mL of ice-cold normal saline.

The liver samples were immediately transferred to the Research Laboratory of Prof. Aghahowa, where they were mechanically homogenised using a mortar and pestle. The homogenates were then buffered with ice-cold 0.25 M sucrose and 10 mM Tris–HCl (pH 7.4), transferred into labelled sample bottles, and stored overnight at -80 °C in a Laboratory Freezer pending microsomal isolation.

First Centrifugation (Removal of Cell Debris and Nuclei)

- The homogenate was thawed and transferred into pre-cooled centrifuge tubes.
- Tubes were balanced and loaded into a refrigerated centrifuge.
- First spin: 4,000 rpm for 1 hour at 4°C. This step pellets nuclei, unbroken cells, and large debris, leaving the post-nuclear supernatant containing microsomes and other subcellular fractions.

Second Centrifugation (Microsome Pelleting)

- The post-nuclear supernatant was carefully decanted into new pre-cooled tubes.
- Second spin: 4,000 rpm for 4 hours at 4 °C. Under these conditions, the microsomal fraction (vesicles derived from the endoplasmic reticulum) sediments to form a pellet at the bottom of the tube.

Resuspension and Storage

The resulting microsomal pellet was gently resuspended in a minimal volume of ice-cold

homogenisation buffer to achieve a concentrated microsomal suspension.

- Aliquots were prepared in pre-labelled sample bottles to avoid repeated freeze-thaw cycles.
- Microsomal aliquots were stored at -80 °C until required for:
    - Protein quantification via the Modified Lowry Method
    - Total CYP450 content determination via the Omura & Sato CO-difference method

Precautions and Quality Control

- All steps were performed on ice or at 4 °C to minimise proteolysis and maintain enzymatic activity.
- Care was taken to avoid disturbing pellets when decanting supernatants.
- Equipment calibration logs were checked to ensure rpm accuracy.

2.2.5 Determination of Microsomal Protein Concentration

The concentration of microsomal protein was determined using the Modified Lowry method, as described by Lowry *et al.* (1951) and adapted for microsomal samples.

Preparation of reagents

All reagents were prepared fresh on the day of analysis using analytical-grade chemicals and distilled water**.** The following solutions were used:

- Bovine serum albumin (BSA) powder (analytical grade)
- Modified Lowry Reagent (Working Solution) - freshly prepared by mixing solution 1, 2, & 3 in a 50:50:1 ratio
    - Solution 1: 4% Na_2CO_3, 0.2 M NaOH, 0.32% potassium sodium tartrate in distilled water

- Solution 2: 2% sodium dodecyl sulphate (SDS) in distilled water
- Solution 3: 4% $CuSO_4 \cdot 5H_2O$ in distilled water

- Folin–Ciocalteu reagent (commercial), diluted 1:1 with distilled water immediately before use

Preparation of BSA Standards (1–1500 µg/mL) and dilution calculations

A 2.00 mg/mL BSA stock solution was prepared by accurately weighing 0.2000 g of BSA and dissolving it with appropriate volume of distilled water to give a 100.0 mL solution. From this stock, a series of standards covering the range 1 µg/mL to 1500 µg/mL was prepared using the dilution formula.

$$C_1 \cdot V_1 = C_2 \cdot V_2$$

$$V_1 = \frac{C_2}{C_1} \cdot V_2$$

where C_1 is stock concentration, V_1 is stock volume to pipette, C_2 is target concentration, and V_2 is final volume.

BSA Standards (1–1500 µg/mL)

Vial	Volume of Stock Solution (mL)	Volume of Distilled Water (mL)	Final Concentration (µg/mL)
A	7.50 of Stock	2.50	1500
B	6.25 of stock	6.25	1000
C	3.10 of vial A	3.10	750
D	6.25 of vial B	6.25	500
E	6.25 of vial D	6.25	250
F	6.25 of vial E	6.25	125
G	2.00 of vial F	8.00	25
H	2.00 of vial G	8.00	5
I	2.00 of vial H	8.00	1
J	0	10.00	0

Sample Preparation

Microsomal aliquots stored at -80 °C were thawed on ice

Assay Procedure

All standards, samples, and blanks were assayed in duplicate of five and an average was determined.

1. Pipetting: 0.8mL of each BSA standard, undiluted liver sample, and blank (distilled water) was transferred into labelled test tubes.
2. Addition of Modified Lowry Reagent: 4.0 mL of freshly prepared Modified Lowry Reagent was added to each tube, mixed thoroughly, and allowed to stand for 10 minutes at room temperature.
3. Addition of Folin-Ciocalteu reagent: 0.4 mL of diluted Folin-Ciocalteu reagent was added rapidly to each tube, followed by immediate and vigorous mixing.
4. Incubation: Tubes were left to stand for 30 minutes at room temperature, protected from direct light, to allow full colour development.
5. Measurement: Absorbance was read at 750 nm using the reagent blank to zero the spectrophotometer.

Calculation of Protein Concentration

A standard curve was generated by plotting the mean absorbance values of the BSA standards against their known concentrations (µg/mL). The protein concentration of each sample was interpolated from this curve, corrected for the dilution factor, and expressed as mg protein/mL of the original microsomal suspension.

The samples used in protein concentration determination were diluted 0.8ml in 5.2ml. Dilution factor was applied and the final concentration adjusted accordingly

$$C_1 \cdot V_1 = C_2 \cdot V_2$$

$$C_1 = \frac{V_2}{V_1} \cdot C_2$$

$$C_1 = \frac{5.2\text{ml}}{0.8\text{ml}} \cdot C_2$$

$$C_1 = 6.5 \times C_2$$

2.2.6 Determination of total CYP450 content

Principle

Total hepatic cytochrome P450 (CYP450) in liver microsomal fractions was quantified using the Omura-Sato carbon monoxide (CO) difference method, adapted to fixed-wavelength reads at 420 nm, 450 nm, and 490 nm on a dual-beam UV-Vis spectrophotometer and mirrored on a microplate reader. Diluted microsomal aliquots were placed in the cuvettes/wells. CO was introduced before chemical reduction with sodium dithionite. After CO exposure and reduction, the reduced CYP450-CO complex exhibited a peak at 450 nm and a trough at 490 nm, while denatured heme (P420) produced a peak at 420 nm. Absorbance differences were converted to amounts using extinction coefficients specific to each species.

Reagents and Materials

- 100 mM potassium phosphate buffer (pH 7.4–7.7) containing:
    - 1.0 mM EDTA
    - 20% (v/v) glycerol
    - 0.5% (w/v) sodium cholate
    - 0.4% (v/v) Triton X-100

- Sodium dithionite -10g weighed into a pre-labelled petri dish
- Carbon monoxide (CO) gas - high-purity grade, delivered via fine-bore tubing
- Quartz cuvettes - 1 cm path length (for UV-Vis)
- UV-Vis spectrophotometer - capable of reading at 420 nm, 450 nm, and 490 nm
- Microplate reader - configured to read at 420 nm, 450 nm, and 490 nm for high-throughput or parallel sample measurement
- Ice bath for maintaining low temperature during preparation and measurement

Sample preparation

For each assay, 0.3 mL of the microsomal sample was diluted into 6.0 mL of freshly prepared assay buffer (100 mM potassium phosphate, pH 7.4-7.7; 1.0 mM EDTA; 20% glycerol; 0.5% sodium cholate; 0.4% Triton X-100). The mixture was gently inverted to ensure homogeneity. No protein concentration targeting was performed; instead, the final CYP450 amount was normalised by the protein concentration previously determined for that sample accounting for the dilution as needed.

- **Buffer preparation:**
    - Phosphate buffer (100 mM, pH 7.4-7.7): Prepared by mixing 100 mM KH_2PO_4 and 100 mM K_2HPO_4 stocks to the desired pH (verified with a calibrated pH meter).
    - EDTA (1.0 mM): Made by diluting a 0.1 M EDTA stock into the buffer.
    - Glycerol (20% v/v): Added as 20 mL per 100 mL of final buffer.
    - Sodium cholate (0.5% w/v): Dissolved at 0.5 g per 100 mL of final buffer.
    - Triton X-100 (0.4% v/v): Added as 0.4 mL per 100 mL of final buffer; mixed thoroughly.

Assay procedure

1. Loading: Aliquots of the diluted sample were transferred into the cuvettes/wells.
2. Baseline flattening: The cuvette was used to zero the absorbance at 420nm, 450nm and 490nm.
3. CO exposure: CO was gently bubbled into the cuvette/well for ~30 seconds under a fume hood, avoiding foaming.
4. Chemical reduction: A small amount of solid sodium dithionite was added to the cuvettes/wells to fully reduce the cytochromes contents were mixed gently.
5. Post-treatment reads: Absorbance were recorded at 450 nm and 490 nm for CYP450 and at 420 nm and 490 nm for P420. All measurements were performed in duplicate of five and an average value was used for calculations.

Identical assay was carried out for microplate measurements.

Calculations

1. Absorbance difference ΔA:

$$\Delta A_{450} = A_{450} - A_{490}$$

$$\Delta A_{420} = A_{420} - A_{490}$$

2. Conversion to nmol P450 per mL was achieved using the extinction coefficient for the reduced CYP450-CO complex (ε= 0.091 ($mL \cdot nmol^{-1} \cdot cm^{-1}$)

$$\frac{\text{nmol } P_{450}}{\text{mL}} = \frac{\Delta A_{450}}{0.091}$$

3. For P420 determination, the same approach was used but substituting 420 nm for 450 nm and applying the extinction coefficient for P420 (ε=0.110)

$$\frac{\text{nmol } P_{420}}{\text{mL}} = \frac{\Delta A_{420}}{0.110}$$

4. The sample used in CYP determination was diluted 0.3ml in 6.3ml. Dilution factor was applied and the final concentration adjusted accordingly.

$$C_1 \cdot V_1 = C_2 \cdot V_2$$

$$C_1 = \frac{V_2}{V_1} \cdot C_2$$

$$C_1 = \frac{6.3\text{ml}}{0.3\text{ml}} \cdot C_2$$

$$C_1 = 21 \times C_2$$

5. Normalisation to protein content was then performed to express results as nmol per mg protein. The protein concentration in the cuvette/well was calculated from the original microsomal protein concentration determined.

   For CYP_{450};

$$\frac{\text{nmol } CYP_{450} \text{ of per ml}}{protein\ conc.\ (mg/ml)} = CYP_{450} nmol/mg$$

   And for CYP_{420};

$$\frac{\text{nmol } CYP_{420} \text{ of per ml}}{protein\ conc.\ (mg/ml)} = CYP_{420} nmol/mg$$

6. The denatured protein was expressed in percentage of the total protein as;

$$\frac{P_{420}}{P_{420} + P_{450}} \times 100 = \%\text{ of Denatured protein}$$

CHAPTER THREE

3.1 Animal Status and Body Weight Changes

Table 3.1: Percentage Weight Change after 14 Days of Treatment (Mean ± SEM)

Group	Dose (mg/Kg)	% Weight Change
Control (Olive oil)	0.5ml	14.2 ± 4.18
Cimetidine (400mg)	6.67	0.9 ± 3.6
Phenobarbital (30mg)	0.50	13.7 ± 2.35
Artemether/Lumefantrine AL (80mg/480mg)	1.33/8.00	20.1 ± 1.85

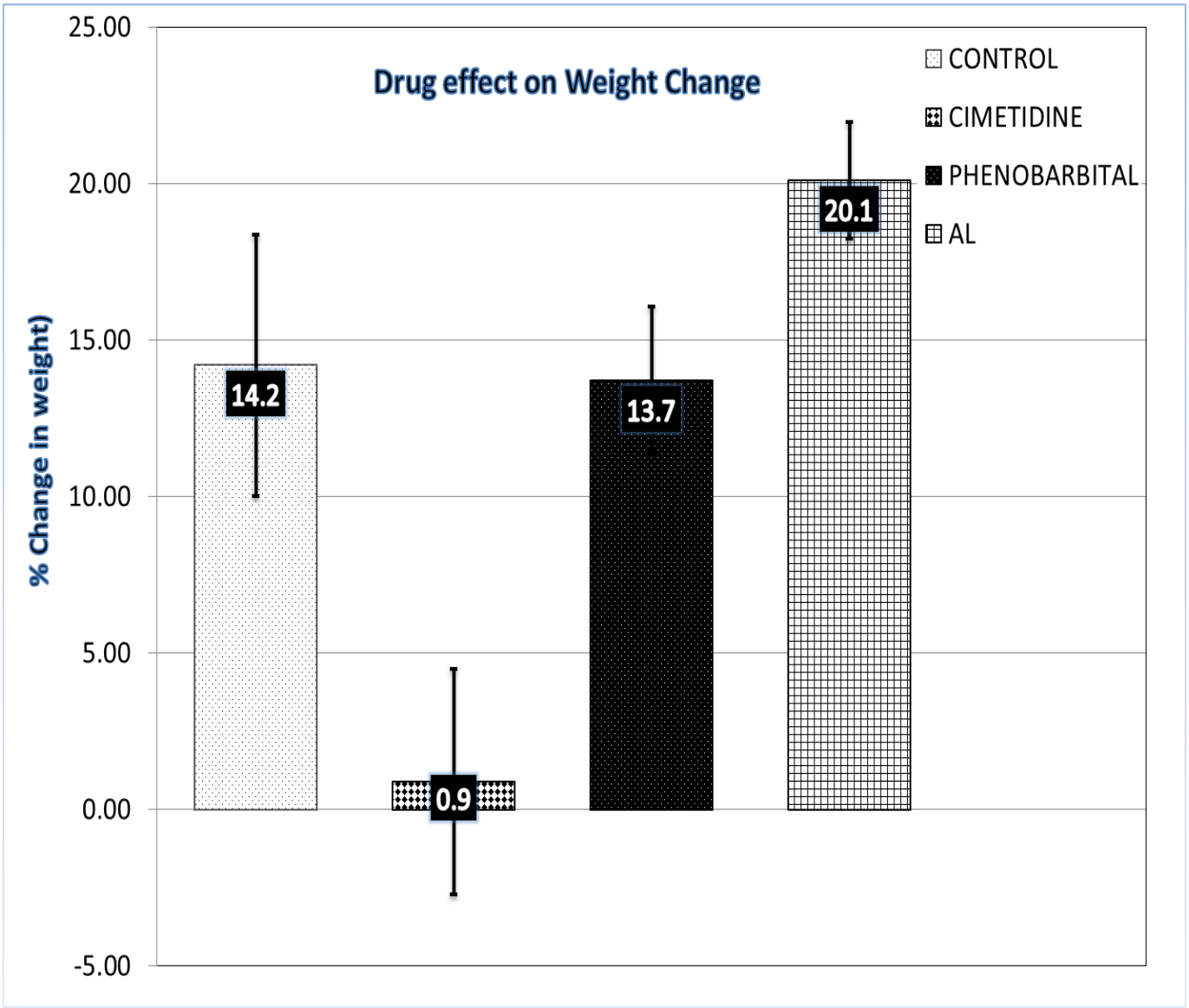

Fig 3.1: Effect of Artemether/Lumefantrine on percentage change in weight in Rat.

3.2 Microsomal Protein Concentration

Table 3.2: Microsomal Protein Concentration (mg/mL, Mean ± SEM)

Group	**Dose (mg/Kg)**	**Protein Concentration (mg/mL)**
Control (Olive oil)	0.5ml	20.92 ± 1.99
Cimetidine (400mg)	6.67	15.83 ± 2.84
Phenobarbital (30mg)	0.50	19.12 ± 2.84
Artemether/Lumefantrine AL (80mg/480mg)	1.33/8.00	16.87 ± 2.20

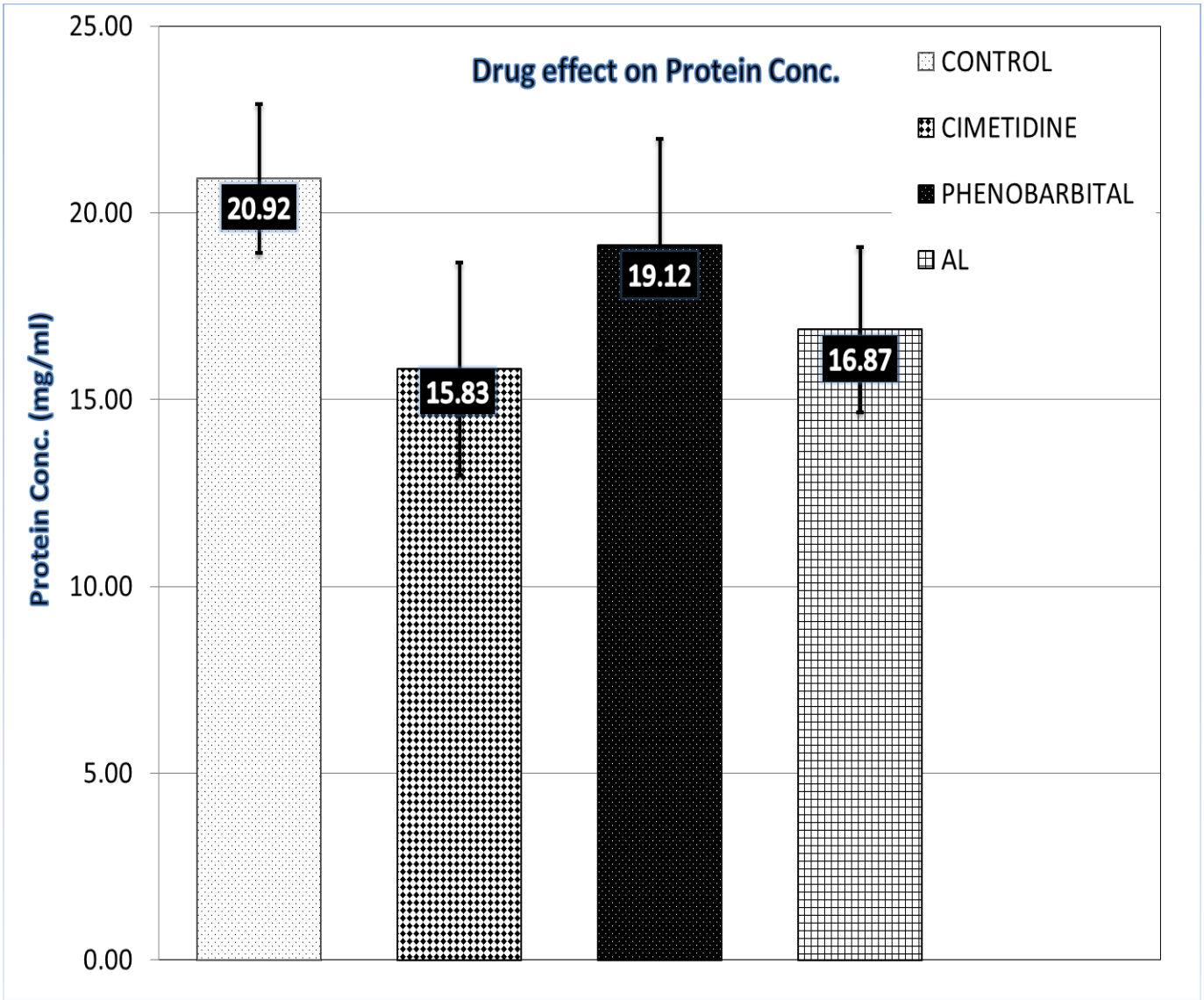

Fig 3.2: Effect of Artemether/Lumefantrine on protein conc. in Ra

3.3 Normalized Cytochrome P450 Content

Table 3.3: Normalized CYP450 Content (nmol/mg protein, Mean ± SEM)

Group	**Dose (mg/Kg)**	CYP450 (nmol/mg protein)
Control (Olive oil)	0.5ml	0.204 ± 0.022
Cimetidine (400mg)	6.67	0.451 ± 0.069
Phenobarbital (30mg)	0.50	0.717 ± 0.186
Artemether/Lumefantrine AL (80mg/480mg)	1.33/8.00	0.165 ± 0.020

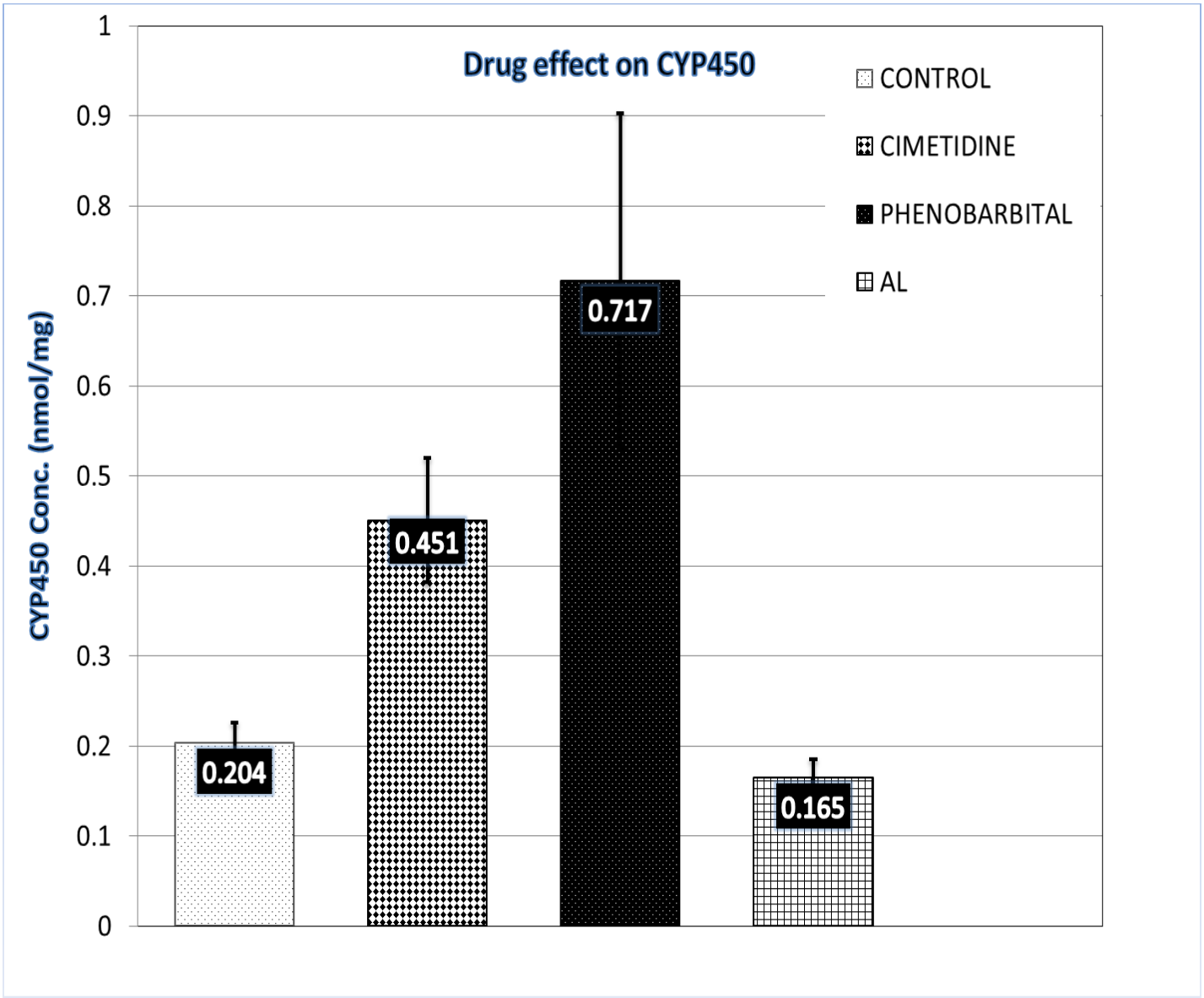

Fig 3.3: Effect of Artemether/Lumefantrine on CYP450 conc. in Rat

Statistical Analysis

- One-way ANOVA revealed a significant difference in normalized CYP450 content among the groups ($p < 0.05$).
- Tukey's HSD post hoc test showed:
    - AL vs Control: Not significantly different
    - AL vs Cimetidine: Significantly different
    - AL vs Phenobarbital: Significantly different

3.4 Denatured Protein (P420)

Table 3.4: Percentage Denatured Protein (Mean ± SEM)

Group	Dose (mg/Kg)	% Denatured Protein
Control (Olive oil)	0.5ml	44.2 ± 0.43
Cimetidine (400mg)	6.67	37.6 ± 0.63
Phenobarbital (30mg)	0.50	34.5 ± 2.95
Artemether/Lumefantrine AL (80mg/480mg)	1.33/8.00	37.7 ± 0.03

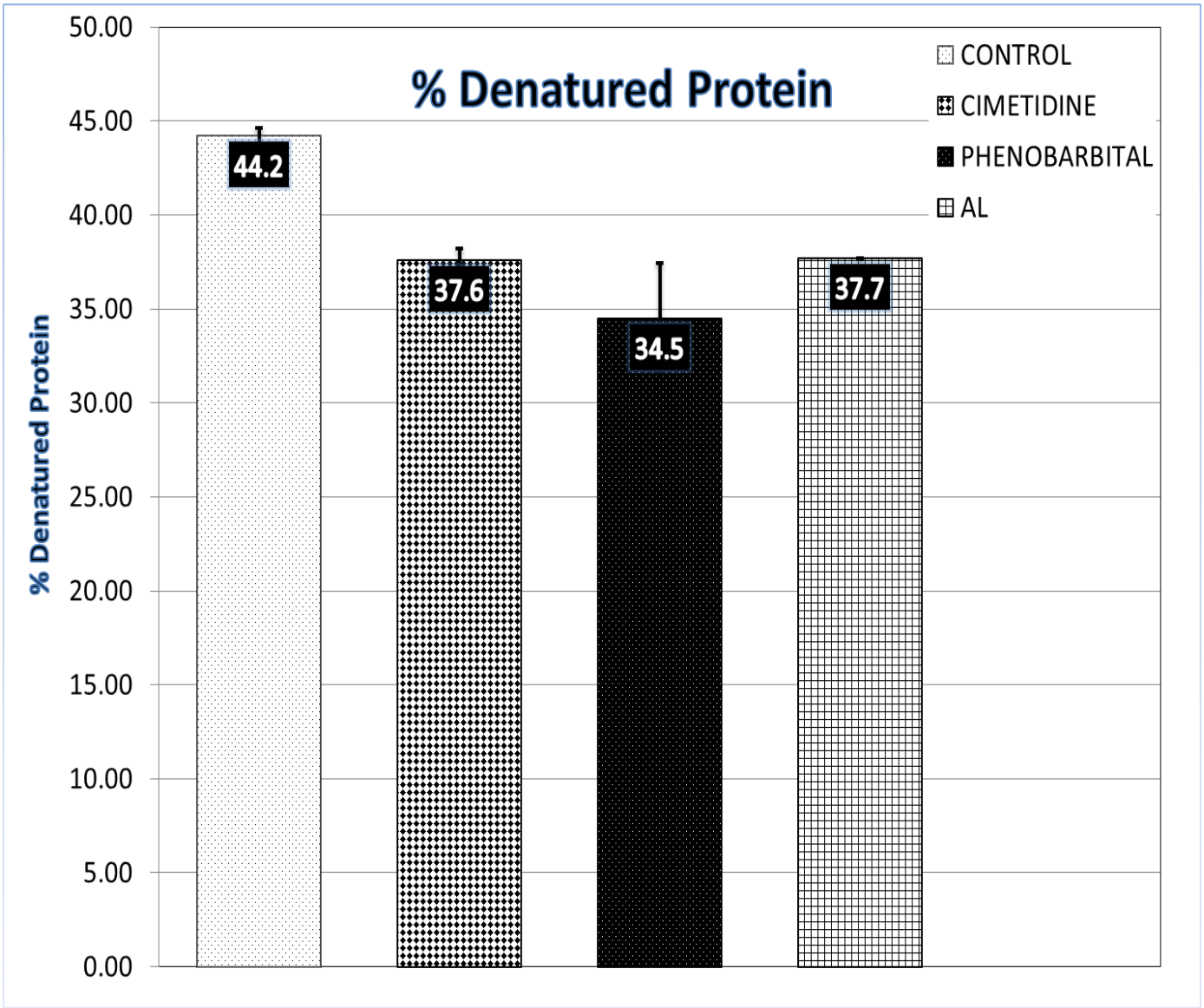

Fig 3.4: Percentage of Denatured Microsomal Protein (P420) Following Freeze–Thaw Cycles during Study

CHAPTER FOUR

4.1 General Overview of CYP450 Modulation

The cytochrome P450 (CYP450) enzyme system plays a central role in the metabolism of xenobiotics, including most antimalarial drugs. These enzymes, located primarily in the liver, are responsible for the oxidative metabolism of a wide range of therapeutic agents, thereby influencing their clearance, bioavailability, and potential for drug–drug interactions (Guengerich, 2021). Because of their broad substrate specificity, CYP450 enzymes are often the first point of contact for orally administered drugs, including Artemether/Lumefantrine (AL).

The importance of this finding lies in its clinical implications. Since AL is widely used as a first-line antimalarial in Nigeria and globally (WHO, 2023), understanding whether it induces or inhibits CYP450 enzymes is essential. If AL acts as an inducer, it could reduce the plasma levels of co-administered drugs, leading to therapeutic failure. Conversely, if it acts as an inhibitor, it could elevate drug levels, increasing the risk of toxicity (Conrad & Rosenthal, 2019). The results of this study therefore provide valuable insights into the potential role of AL as a modulator of CYP450 enzymes.

4.2 Inherent Inductive Properties of Test Drugs

One of the striking observations from this study is that all drug-treated groups (cimetidine, phenobarbital, and AL) showed relatively higher CYP450 concentrations compared to the olive oil control, though it wasn't very visible for the AL group because of it inhibitory effect. This pattern is not unexpected when viewed through the lens of hepatic pharmacology.

Any compound that is metabolised by the CYP450 system acts as a substrate, and the liver responds to the presence of such xenobiotics by upregulating enzyme production in an attempt to enhance clearance (Henderson *et al.* 2019). This adaptive response is sometimes referred to as a minute inherent inductive property of substrates. It does not necessarily mean that the drug is a true inducer in the pharmacological sense, but rather that the body is mounting a physiological response to the foreign compound.

This explains why even cimetidine, a well-established CYP450 inhibitor, still demonstrated a higher CYP450 concentration than olive oil. The same applies to phenobarbital, which is both a

substrate and a potent inducer, and to AL, which is metabolised by CYP3A4 and therefore stimulates enzyme production to some degree (Conrad & Rosenthal, 2019; Guengerich, 2021).
The key point here is that olive oil serves as the true baseline, since it is not a CYP substrate and therefore does not trigger enzyme up regulation. By contrast, all drug groups inherently show higher values because of their substrate nature. This provides the context for interpreting the relative positions of AL, cimetidine, and phenobarbital in the results chart.

4.3 The Cimetidine Anomaly

One of the most notable findings in this study was the unexpected elevation of CYP450 concentration in the cimetidine group**.** Cimetidine is widely recognised as a CYP450 inhibitor, particularly of the CYP3A4 isoform, and is often used as a reference negative control in enzyme modulation studies (FDA, 2020). Its inhibitory mechanism, however, is not based on reducing the concentration of CYP enzymes, but rather on reversibly binding to the active site of the enzyme and blocking its catalytic activity (Henderson *et al.* 2019).
This distinction between enzyme activity and enzyme concentration is crucial for interpreting the results. While cimetidine effectively inhibits the metabolic activity of CYP450 enzymes, the liver still recognizes it as a foreign substrate. In response, the liver attempts to increase enzyme production to facilitate clearance. The result is a paradox: higher measured CYP450 concentration despite reduced enzymatic activity**.**
This explains why the cimetidine group showed a significant rise in CYP450 concentration compared to the olive oil control. The apparent contradiction is resolved when one considers that the Omura & Sato CO-difference method used in this study measures total enzyme concentration, not functional activity. Thus, the elevated concentration does not contradict cimetidine's role as an inhibitor; rather, it reflects the liver's compensatory response to the drug's presence.
For this reason, cimetidine remains a valid negative control in CYP450 studies. It provides a benchmark for inhibition, even though its measured concentration may appear elevated. The true inhibitory effect lies in its ability to reduce catalytic activity, not in lowering enzyme levels (Conrad & Rosenthal, 2019).

4.4 Rationale for Control Groups

The interpretation of CYP450 modulation in this study relies heavily on the appropriate use of control groups. Two distinct controls were employed: cimetidine as the negative control and olive oil as the inhibitory control**.**

Cimetidine as the negative control: Although cimetidine is classically described as a CYP450 inhibitor, in this experimental context it is best understood as a negative control. Like other drug substrates, cimetidine exhibits the general inherent inductive property of stimulating the liver to produce more enzymes in an attempt to clear the xenobiotic (Henderson *et al.* 2019). However, beyond this baseline substrate effect, cimetidine does not exert a true inductive or inhibitory influence on CYP concentration. Thus, its role here is to represent the "no effect" condition, a benchmark against which the test drug (AL) can be compared. If AL behaves similarly to cimetidine, it can be concluded that AL has no significant modulatory effect on CYP450 beyond the inherent substrate response.

Olive oil as the inhibitory control: Olive oil, on the other hand, does not act as a substrate for CYP450 metabolism and therefore does not trigger the inherent inductive effect. This makes it a better inhibitory control, as it reflects the lowest baseline enzyme concentration (National Research Council, 2011). Any CYP substrate drug that aligns closely with olive oil in normalized CYP450 content even with its inherent inductive effect can be interpreted as exhibiting an inhibitory effect, since its inherent inductive property has been effectively cancelled out leaving it at the same level as the non-substrate control.

Why both controls are necessary: The dual-control design provides a nuanced framework for interpretation. Cimetidine establishes the neutral baseline (no effect beyond substrate induction), while olive oil establishes the inhibitory baseline (absence of induction). By comparing AL to both, it becomes possible to classify its effect more precisely.

4.5 Artemether/Lumefantrine (AL) as a Potential Inhibitor

The results of this study demonstrate that Artemether/Lumefantrine (AL) closely aligned with the olive oil group in normalized CYP450 content, rather than with the cimetidine group. This distinction is critical for interpreting AL's pharmacological effect.

As explained earlier, cimetidine serves as the negative control, representing the baseline substrate response without true modulation of CYP450 concentration. In contrast, olive oil serves

as the inhibitory control, since it does not exhibit the inherent inductive effect seen with drug substrates. Therefore, any CYP substrate test drug that produces values similar to olive oil can be classified as having an inhibitory effect, because its inherent inductive property has been effectively neutralised.

In this study, AL's normalized CYP450 concentration was not significantly different from olive oil but was significantly lower than both cimetidine and phenobarbital. This indicates that AL's inherent substrate-driven induction was cancelled out, leaving it at the same level as the inhibitory control. Thus, AL can be classified as a net inhibitor of CYP450 enzymes**.**

This finding has important clinical implications. Since AL is metabolised by CYP3A4, its inhibitory effect suggests that it may slow the metabolism of co-administered drugs, particularly those that are also CYP3A4 substrates. This could result in elevated plasma concentrations of such drugs, increasing the risk of adverse effects or toxicity (Conrad & Rosenthal, 2019; WHO, 2023). This is especially relevant in malaria-endemic regions like Nigeria, where AL is frequently co-prescribed with antiretrovirals, antituberculosis agents, and other essential medicines.

By behaving more like the inhibitory control (olive oil) than the negative control (cimetidine), AL demonstrates that it is not pharmacologically neutral with respect to CYP450. Instead, it actively contributes to the risk of drug–drug interactions by functioning as an inhibitor. This aligns with the broader concern that ACTs, while effective antimalarial may complicate therapy in patients on multiple medications (German *et al.* 2016).

4.6 Clinical and Pharmacological Implications

The classification of Artemether/Lumefantrine (AL) as a potential inhibitor of CYP450 enzymes carries important clinical and pharmacological consequences. Since AL is the most widely used first-line artemisinin-based combination therapy (ACT) in Nigeria and many other malaria-endemic regions (WHO, 2023), its interaction with hepatic enzymes has direct implications for patient safety and therapeutic outcomes.

Impact on drug–drug interactions: If AL inhibits CYP450 activity, it may slow the metabolism of co-administered drugs, particularly those metabolised by CYP3A4, such as antiretrovirals, antituberculosis agents, and certain cardiovascular medications (Conrad & Rosenthal, 2019). This could lead to elevated plasma concentrations of these drugs, increasing

the risk of adverse effects or toxicity. For example, patient co-infected with malaria and HIV or tuberculosis, a common scenario in sub-Saharan Africa, may be especially vulnerable to such interactions.

Polypharmacy and therapeutic safety: In malaria-endemic regions, polypharmacy is frequent due to overlapping infectious and chronic diseases. The inhibitory effect of AL suggests that clinicians should exercise caution when prescribing it alongside other essential medicines. Monitoring for signs of toxicity, dose adjustments, or alternative therapies may be necessary in high-risk patients (German *et al.* 2016).

Public health relevance: At the population level, the inhibitory profile of AL underscores the need for pharmacovigilance systems to track adverse drug reactions and treatment failures. Since ACTs are central to malaria control strategies, understanding their interaction potential is vital for sustaining their effectiveness and ensuring patient safety.

Future research directions: While this study provides strong evidence of AL's inhibitory effect, further research is needed to:

- Confirm these findings using functional enzyme activity assays, not just concentration measurements.
- Explore the impact of AL on specific CYP isoforms (e.g., CYP3A4, CYP2B6).
- Investigate alternative inhibitors (e.g., clarithromycin) as controls to validate the classification framework.
- Conduct clinical pharmacokinetic studies in humans to assess the real-world magnitude of AL's inhibitory effect.

4.7 Relevance of Other Results

Although the primary focus of this study was the effect of Artemether/Lumefantrine (AL) on normalized CYP450 content, the additional parameters measure; Denatured protein (P420), microsomal protein concentration, and percentage weight change provide essential context for validating the reliability and clinical significance of the findings.

Denatured Protein (P420): The determination of denatured protein was carried out to assess the integrity of microsomal samples and to establish whether freeze-thaw cycles during the three-week study compromised the reliability of results. Denaturation arises naturally from repeated freezing and thawing, which can deteriorate protein structure. In this study, the

percentage of denatured protein ranged between 34% and 45% across groups. This indicates that more than half of the microsomal protein remained intact and functional, ensuring that the CYP450 assays were conducted on sufficiently preserved samples. Thus, despite the unavoidable freeze-thaw effect, the results are robust and reliable. For AL specifically, the denatured protein value (37.7%) confirms that its apparent inhibitory effect on CYP450 concentration was not due to sample degradation, but rather a true pharmacological outcome.

Microsomal Protein Concentration: Protein concentration was measured primarily to enable normalization of CYP450 content, ensuring that enzyme levels were expressed relative to protein mass rather than raw absorbance values. This step is critical for accurate comparison across groups. The results showed that AL (16.87 mg/mL) and cimetidine (15.83 mg/mL) groups had lower protein concentrations compared to control (20.92 mg/mL), while phenobarbital was intermediate (19.12 mg/mL). These values confirm that the liver maintained adequate protein output during treatment, even though AL reduced protein concentration relative to control. Importantly, protein concentration can be influenced by several external and internal factors beyond drug treatment. Environmental factors such as diet quality, stress, ambient temperature, and housing conditions, as well as genetic variability in protein synthesis capacity, may all contribute to differences in protein concentration.

Percentage Weight Change: Body weight change was monitored as a general indicator of animal health and drug tolerability. AL produced the highest weight gain (20.1%), control (14.2%) and phenobarbital (13.7%) groups showed moderate increases, while cimetidine exhibited minimal change (0.9%) with one mortality recorded. The noticeable reduction in weight in the cimetidine group may be explained by its pharmacological action: cimetidine reduces gastric acidity, which can impair digestion and nutrient absorption, ultimately limiting weight gain. As with protein concentration, weight change is also subject to environmental influences such as food availability, cage conditions, and stress, as well as genetic factors including metabolic rate and growth potential.

Overall Relevance: Together, these supporting parameters strengthen the interpretation of the main findings. The denatured protein analysis confirms that the study results are reliable despite freeze-thaw cycles. Protein concentration measurements validate the normalization of CYP450 content while highlighting possible environmental and genetic influences. Weight change data provide evidence of tolerability, with the cimetidine anomaly explained by its known

pharmacological effects. Collectively, these results demonstrate that AL exerts a neutral to inhibitory effect on CYP450 concentration without systemic toxicity, reinforcing the clinical relevance of the study.

CHAPTER FIVE

5.1 Conclusion

This study showed that Artemether/Lumefantrine (AL) acts as a net inhibitor of hepatic CYP450 enzymes in albino rats. While all drug-treated groups exhibited the general inherent inductive effect of substrates, AL's normalized CYP450 content was closest to olive oil (the inhibitory control) rather than cimetidine (the negative control). This indicates that AL's inductive effect was neutralised, leaving it inhibitory overall. Supporting results (denatured protein, protein concentration, and weight change) confirmed that AL's effect was specific to CYP450 modulation and not due to systemic toxicity. Clinically, this suggests AL may slow the metabolism of co-administered drugs, raising the risk of drug accumulation and toxicity in polypharmacy settings.

5.2 Recommendations

- **Clinical practice:** Prescribers should exercise caution when combining AL with other CYP450 substrates (e.g., antiretrovirals, TB drugs, cardiovascular agents). Monitoring for toxicity is advised in patients on multiple medications.
- **Research:** Future studies should measure enzyme activity (not just concentration), investigate isoform-specific effects (e.g., CYP3A4), and validate findings with alternative inhibitors such as clarithromycin.
- **Public health:** Strengthen pharmacovigilance systems to detect AL-related drug interactions and update malaria treatment guidelines to highlight its inhibitory potential.

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APPENDICES

Animal Status and Weight Changes

Rat ID	Weight at 0	Weight at 15	Weight Change	% change in weight	Status
OIL-R1	156.4	185.7	29.3	18.7%	Survived
OIL-R2	203.1	212.2	9.1	4.5%	Survived
OIL-B1	153.1	201.1	48.0	31.4%	Survived
OIL-B2	174.1	183.2	9.1	5.2%	Survived
OIL-G1	214.5	233.7	19.2	9.0%	Survived
OIL-G2	165.0	192.1	27.1	16.4%	Survived
CIM-R1	161.2	166.6	5.4	3.3%	Survived
CIM-R2	236.5	-	-		Died on day 12
CIM-B1	209.7	183.3	-26.4	-12.6%	Survived
CIM-B2	181	184.6	3.6	2.0%	Survived
CIM-G1	202.2	220.4	18.2	9.0%	Survived
CIM-G2	171.9	177.1	5.2	3.0%	Survived
PHB-R1	153.4	173.7	20.3	13.2%	Survived
PHB-R2	146.3	167.3	21.0	14.4%	Survived
PHB-B1	164.0	220.3	36.3	22.1%	Survived
PHB-B2	145.5	166.7	21.2	14.6%	Survived
PHB-G1	146.2	152.1	5.9	4.0%	Survived
PHB-G2	158.6	180.6	22.0	13.9%	Survived
AL-R1	157.9	192.4	34.5	21.8%	Survived

AL-R2	152.3	195.8	43.5	28.6%	Survived
AL-B1	155.6	182.6	27	17.4%	Survived
AL-B2	130.7	153.3	22.6	17.3%	Survived
AL-G1	172.5	205.3	32.8	19.0%	Survived
AL-G2	146.2	170.6	24.4	16.7%	Survived

Microsomal Protein Concentration

Rat ID	**Mean Absorbance at 750nm**	**Initial Concentration** C_2 **(mg/mL)**	**Final Concentration** C_1 **(mg/mL)**
OIL-R1	0.424	3.95	25.68
OIL-R2	0.429	4.02	26.13
OIL-B1	0.398	3.27	21.26
OIL-B2	0.312	2.31	15.02
OIL-G1	0.388	3.42	22.23
OIL-G2	0.314	2.34	15.21
CIM-R1	0.352	2.89	18.79
CIM-R2	-	-	-
CIM-B1	0.455	4.32	28.10
CIM-B2	0.408	3.71	24.12
CIM-G1	0.252	1.43	9.30
CIM-G2	0.337	2.67	17.36
PHB-R1	0.400	3.59	23.34
PHB-R2	0.273	1.74	11.31

PHB-B1	0.393	3.49	22.69
PHB-B2	0.460	4.47	29.06
PHB-G1	0.290	1.98	12.87
PHB-G2	0.317	2.38	15.47
AL-R1	0.298	2.10	13.65
AL-R2	0.436	4.12	26.78
AL-B1	0.288	1.95	12.68
AL-B2	0.336	2.66	17.29
AL-G1	0.345	2.79	18.14
AL-G2	0.288	1.95	12.68

CYP450 CO-difference assay

Rat ID	$\mathbf{A_{450}}$	$\mathbf{A_{490}}$	$\mathbf{A_{450-490}}$	C_2 CYP_{450} ($nmol/ml$)	C_1 CYP_{450} ($nmol/ml$)	*Protein conc. (mg/ml)*	CYP_{450} $conc.$ (**nmol/mg**)
OIL-R1	0.112	0.095	0.017	0.187	3.927	25.68	0.153
OIL-R2	0.105	0.088	0.017	0.187	3.927	26.13	0.150
OIL-B1	0.134	0.116	0.018	0.198	4.158	21.26	0.196
OIL-B2	0.091	0.073	0.018	0.198	4.158	15.02	0.277
OIL-G1	0.113	0.095	0.018	0.198	4.158	22.23	0.187

OIL-G2	0.118	0.101	0.017	0.187	3.927	15.21	0.258
CIM-R1	0.146	0.112	0.034	0.374	7.854	18.79	0.418
CIM-R2	-	-	-	-	-	-	-
CIM-B1	0.160	0.119	0.041	0.451	9.471	28.10	0.337
CIM-B2	0.135	0.106	0.029	0.319	6.699	24.12	0.278
CIM-G1	0.121	0.097	0.024	0.264	5.544	9.30	0.596
CIM-G2	0.171	0.124	0.047	0.516	10.836	17.36	0.624
PHB-R1	0.180	0.119	0.061	0.670	14.07	23.34	0.603
PHB-R2	0.176	0.134	0.042	0.462	9.702	11.31	0.858
PHB-B1	0.169	0.135	0.034	0.374	7.854	22.69	0.346
PHB-B2	0.199	0.184	0.015	0.165	3.465	29.06	0.119
PHB-G1	0.114	0.088	0.056	0.615	12.915	12.87	1.003
PHB-G2	0.271	0.179	0.092	1.011	21.231	15.47	1.372
AL-R1	0.161	0.148	0.013	0.143	3.000	13.65	0.220
AL-R2	0.171	0.159	0.012	0.132	2.769	26.78	0.103
AL-B1	0.154	0.142	0.012	0.132	2.769	12.68	0.218
AL-B2	0.156	0.145	0.011	0.121	2.538	17.29	0.147
AL-G1	0.158	0.144	0.014	0.154	3.231	18.14	0.178
AL-G2	0.164	0.101	0.006	0.073	1.538	12.68	0.121

Denatured P450 (P420) results and denaturation percentage

Rat ID	A_{420}	A_{490}	$A_{420-490}$	C_2 CYP_{420} $(nmol/ml)$	C_1 CYP_{420} $(nmol/ml)$	*Protein conc. (mg/ml)*	CYP_{420} *conc.* $(nmol/mg)$	$\frac{P_{420}}{P_{450}+P_{420}}\%$
OIL-R1	0.118	0.095	0.0017	0.153	3.205	25.68	0.125	44.9%
OIL-R2	0.109	0.088	0.0015	0.139	2.927	26.13	0.112	42.7%
OIL-B1	0.139	0.116	0.0017	0.153	3.205	21.26	0.151	43.5%
OIL-B2	0.097	0.073	0.0018	0.159	3.345	15.02	0.223	44.6%
OIL-G1	0.120	0.095	0.0018	0.166	3.484	22.23	0.157	45.6%
OIL-G2	0.123	0.101	0.0016	0.146	3.066	15.21	0.202	43.9%
CIM-R1	0.147	0.112	0.0026	0.232	4.878	18.79	0.260	38.3%
CIM-R2	-	-	-	-	-	-	-	-
CIM-B	0.16	0.11	0.0030	0.272	5.714	9.56	0.598	37.6%

1	0	9						
CIM-B 2	0.13 3	0.10 6	0.0020	0.179	3.763	24.12	0.156	35.9%
CIM-G 1	0.12 3	0.09 7	0.0019	0.173	3.623	9.30	0.390	39.5%
CIM-G 2	0.16 9	0.12 4	0.0033	0.299	6.271	17.36	0.361	36.7%
PHB-R 1	0.18 2	0.11 9	0.0046	0.418	8.780	23.34	0.376	38.45
PHB-R 2	0.18 0	0.13 4	0.0034	0.305	6.411	11.31	0.567	39.8%
PHB-B 1	0.16 9	0.13 5	0.0025	0.226	4.738	22.69	0.209	37.6%
PHB-B 2	0.19 9	0.18 4	0.0011	0.100	2.090	29.06	0.072	37.7%
PHB-G 1	0.11 2	0.08 8	0.0018	0.159	3.345	12.87	0.260	20.6%
PHB-G 2	0.25 3	0.17 9	0.0054	0.491	10.313	15.47	0.667	32.7%
AL-R1	0.16 1	0.14 8	0.0009	0.086	1.812	13.65	0.133	37.7%
AL-R2	0.17 1	0.15 9	0.0009	0.080	1.672	26.78	0.062	37.6%

AL-B1	0.154	0.142	0.0009	0.080	1.672	12.68	0.132	37.7%
AL-B2	0.156	0.145	0.0008	0.073	1.533	17.29	0.089	37.7%
AL-G1	0.158	0.144	0.0010	0.093	1.951	18.14	0.108	37.8%
AL-G2	0.164	0.101	0.0046	0.418	8.780	12.68	0.692	37.6%


PHOTO 750.0 nm 0.006 Abs
No. Abs K = Abs
Press START to measure