THE EFFECT OF QUININE-ARTESUNATE CO-ADMINISTRATION ON SOME BIOCHEMICAL PARAMETERS IN *PLASMODIUM BERGHEI* PARASITIZED SWISS ALBINO MICE AND RATS

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MAY 2014

DECLARATION

I declare that the work in this thesis entitled "THE EFFECT OF QUININE-ARTESUNATE CO-ADMINISTRATION ON SOME BIOCHEMICAL PARAMETERS IN *PLASMODIUM BERGHEI* PARASITIZED SWISS ALBINO MICE AND RATS" has been carried out by me in the Department of Pharmacology and Therapeutics. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree or diploma at this or any other institution.

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Name of Student	Signature	Date

CERTIFICATION

This thesis entitled "THE EFFECT OF QUININE-ARTESUNATE CO-ADMINISTRATION ON SOME BIOCHEMICAL PARAMETERS IN *PLASMODIUM BERGHEI* PARASITIZED SWISS ALBINO MICE AND RATS" by RABIAH SHEHU BIDA meets the regulations governing the award of the degree of Master of Science in Pharmacology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Malaria has been the leading cause of morbidity and mortality in most Sub-saharan Africa and Southeastern Asia. Combination therapy has been recommended for treatment of malaria and this could lead to drug-drug interaction. This work titled "The effect of Quinine-Artesunate co-administration on some biochemical parameters on healthy and *Plasmodium berghei* parasitized Swiss albino mice and rats" was carried out to investigate the effect of Quinine (60 mg/kg)-Artesunate (30 mg/kg) co-administration following 7 days pre treatment. Mice were infected with *Plasmodium berghei* NK65 strain. Parasitaemia was checked after Geimsa stain and viewed for infected blood cells. Five days of establishment of the infection, they were treated for 7 days. The effect on blood glucose level was evaluated using the glucose oxidase principle; the Liver functions and Oxidative stress were assessed using colorimetric kits. The fasting blood glucose and oral glucose tolerance test of the healthy animals were also checked. The study also evaluated the effect of Quinine-Artesunate co-administration on acetic acid induced algesia in mice; while brewer's yeast induced pyrexia was carried out in rats. The effect of the single drug (Quinine or Artesunate) on pain and pyrexia was compared with concurrent administration of quinine and Ibuprofen (100 mg/kg) or artesunate and Ibuprofen. Results of Quinine-Artesunate co-administration did not show any significant reduction in glucose level compared to normal control, infected control, quinine and artesunate groups. The co-administration did not show any significant increase in liver enzymes compared to the other groups. This is in contrast to quinine group that showed significant increase ($P<0.01$) in liver enzyme (ALT) compared to normal control group of parasitized animal's study. The quinine group also showed significant increase in AST

level in healthy animals compared to Artesunate group ($P<0.05$). Quinine-Artesunate co-administration did not show significant difference in lipid peroxidation and antioxidant enzymes biomarkers in healthy animals compared to quinine, artesunate and the normal control groups. It showed less significant increase in lipid peroxidation (MDA) compared to normal control group ($P<0.05$) than quinine ($P<0.01$) and artesunate ($P<0.01$) groups. Quinine-Artesunate group also showed a lesser significant decrease ($P<0.01$) in antioxidant enzyme (CAT) compared to normal control group than artesunate and quinine groups ($P<0.0001$). Quinine-Artesunate group did not show significant difference in antioxidant enzymes compared to the infected control group. In healthy animals, Quinine-Artesunate did not show significant difference in organ body weight ratio between the groups but showed significant decrease in liver-body weight ratio in parasitized animals compared to the infected control group ($P<0.0001$). However, no significant difference was seen in liver-body weight ratio when it was compared to the normal control group as seen in the quinine, artesunate and infected control groups. A significant decrease was also seen in Quinine-Artesunate group compared to the quinine group ($P<0.01$) in the spleen-body weight ratio. No significant difference was seen in haematological parameters of healthy animals in Quinine-Artesunate group compared to other groups. The Quinine-Artesunate group did not show significant difference during concurrent administration of Ibuprofen in pain and pyrexia.

This study however, showed that Quinine and Artesunate are better in efficacy and safety when co-administered and does not reduce the efficacy of ibuprofen during pain and pyrexia.

TABLE OF CONTENTS

Title Page i

DECLARATION ii

CERTIFICATION iii

ACKNOWLEDGEMENTS iv

ABSTRACT v

TABLE OF CONTENTS vii

LIST OF TABLES xii

LIST OF FIGURES xiv

LIST OF APPENDICES xv

ABBREVIATIONS, DEFINITIONS, GLOSSARY AND SYMBOLS xvi

CHAPTER ONE 1

1.0 INTRODUCTION 1

1.1 Preamble 1

1.2 Statement of Research Problem 2

1.3 Justification 3

1.4 Aim 5

1.5 Hypothesis 5

CHAPTER TWO 6

2.0 LITERATURE REVIEW 6

2.1 Malaria 6

2.2 Prevalence of Malaria 6

2.3 Transmission 7

2.4 Signs and Symptoms of Malaria 8

2.5 Classification of Malaria 8

2.6 Diagnosis of Malaria 9

2.7 Life cycle of Malaria Parasite 10

2.7.1 The Asexual Erythrocytic Phase in Humans 10

2.7.2 The Sexual Stage in Mosquito 11

2.8 Malaria and Glucose Metabolism 12

2.9 Oxidative Stress in Malaria 13

2.10 Treatment of Malaria 14

2.11 Quinine 17

2.11.1 Chemistry 18

2.11.2 Pharmacokinetics 18

2.11.3 The Mechanism of Action of Quinine 19

2.11.4 Resistance 20

2.11.5 Therapeutic uses 20

2.11.6 Adverse Effects 22

2.11.7 Contraindications and Drug Interactions 23

2.12 Artesunate: Artemisinin Derivative 23

2.12.1 Mechanism of Action of Artesunate 26

2.12.2 Combination Therapy 26

2.13 Ibuprofen 27

2.14 Drug-Drug Interaction 28

2.14.1 Quinine-Artesunate Combination 29

CHAPTER THREE 31

3.0 MATERIALS AND METHODS 31

3.1 Laboratory Animals 31

3.2 Chemicals, Drugs, Reagents and other Materials 31

3.3 Preparation of Drugs 32

3.4 Study Design 33

3.5 Malaria Parasitized Animals 34

3.6 Glucose Level on Healthy Animals (Uninfected mice) 34

3.6.1 Oral Glucose Tolerance Test 34

3.7 Biochemical Parameters Analysis 35

3.7.1 Liver Function Tests 36

3.7.2 Oxidative Stress Biomarkers 37

3.8 Haematological Parameters Analysis 38

3.9 Organ Body Weight Ratio 38

3.10 Analgesic Study: Acetic Acid Induced Abdominal Constrictions 38

3.11 Anti-Pyretic Study: Brewer's Yeast Induced Pyrexia Model 39

3.12 Statistical Analysis 39

CHAPTER FOUR 41

4.0 RESULTS 41

4.1 The Effect of Quinine, Artesunate, Quinine-Artesunate Co-administration and their Separate Therapy on Blood Glucose Level in Swiss Albino Mice 41

4.2 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Oral Glucose Tolerance Test in Swiss Albino Mice 42

4.3 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Liver Function Test of Albino Mice 44

4.4 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Oxidative Stress Biomarkers on Swiss Albino Mice 45

4.5 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Hematological Parameters in Swiss Albino Mice 46

4.6 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Relative Organ Body Weight in Swiss Albino Mice 47

4.7 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Random Blood Glucose Level in *P. berghei* Infected Mice 48

4.8 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Fasting Blood Glucose Level in *P. berghei* infected mice 49

4.9 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Liver Function Test in *Plasmodium berghei* Infected Mice 50

4.10 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Oxidative Stress Biomarkers in *P. berghei* Infected Mice.....51

4.11 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Relative Organ Body Weight of *P. berghei* Infected Mice.....53

4.12 The Effect of Quinine-Artesunate Co-administration on Acetic Acid Induced Writhes in Mice.....55

4.13 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Brewer's Yeast Induced Pyrexia in Rats.....56

CHAPTER FIVE 58

5.0 DISCUSSIONS 58

CHAPTER SIX.....65

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS 65

6.1 Summary 65

6.2 Conclusion.....65

6.3 Recommendations 66

REFERENCES 68

APPENDIX.....80

LIST OF TABLES

Table 3.1 Experimental Design of Drug Administration in the Animals.....33

Table 3.2 Experimental Design of Drug Administration in the Uninfected Mice.....33

Table 3.3 Experimental Design of Drug Administration in Algesic Mice and Pyretic Rats.....33

Table 4.1 The Effect of Quinine-Artesunate Co-administration on Fasting Blood Glucose Level in Swiss Albino Mice……..……. 41

Table 4.2 The Effect of Quinine-Artesunate Co-administration on Oral Glucose Tolerance Test in Swiss Albino Mice…….....….. 43

Table 4.3 The Effect of Quinine-Artesunate Co-administration on Liver Function Test in Swiss Albino Mice ……..….....…… 44

Table 4.4 The Effect of Quinine-Artesunate Co-administration on Oxidative Stress Biomarkers Test in Swiss Albino Mice.....…..……. 45

Table 4.5 The Effect of Quinine-Artesunate Co-administration on Haematological Indices in Swiss Albino Mice.....…….…….. 46

Table 4.6 The Effect of Quinine-Artesunate Co-administration on Relative Organ Body Weight in Swiss Albino Mice…….....…… 47

Table 4.7 The Effect of Quinine-Artesunate Co-administration on Random Blood Glucose Level in *P. berghei* Infected Mice…...….....……..... 48

Table 4.8 The Effect of Quinine-Artesunate Co-administration on Fasting Blood Glucose Level in *P. berghei* Infected Mice…….……. 49

Table 4.9 The Effect of Quinine-Artesunate Co-administration on Liver Function Test in *P. berghei* Infected Mice…….... 50

Table 4.10 The Effect of Quinine-Artesunate Co-administration on Oxidative Stress Biomarkers Test in *P. berghei* Infected Mice 52

Table 4.11 The Effect of Quinine-Artesunate Co-administration on Relative Organ Body Weight of *P. berghei* Infected Mice 54

Table 4.12 The Effect of Quinine-Artesunate Co-administration on Brewer's Yeast Induced Pyrexia in Wistar Albino Rats 57

LIST OF FIGURES

Figure 2.1 Pictorial Representation of Malaria Parasite Life Cycle.....12

Figure 4.1 The Effect of Quinine-Artesunate Co-administration on Acetic Acid Induced Writhes in Mice……...…… 55

LIST OF APPENDICES

Appendix 1: The Effect of Quinine-Artesunate Co-administration on Acetic Acid Induced Writhes in Mice……...….80

Appendix 2: The effect of Quinine-Artesunate Co-administration on Brewer's Yeast Induced Pyrexia in Albino Rats.....81

Appendix 3: Contents of the Pelletised Growers Feed.....82

ABBREVIATIONS, DEFINITIONS, GLOSSARY AND SYMBOLS

ABUTH	Ahmadu Bello University Teaching hospital
ACT	Artemisinin-based Combination Therapy
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase/Alanine Amino Transferase
ANOVA	Analysis of variance
AST	Aspartate Transaminase/Aspartate Amino Transferase
CAT	catalase
COX	cyclo oxygenase
DNA	deoxyribonucleic acid
FBS/ FBG	fasting blood sugar/fasting blood glucose
g/dL	grams per deciliter
G6PD	glucose-6-phosphate dehydrogenase
GOT	Glutamic Oxaloacetic Transaminase
GPT	Glutamic Pyruvic Transaminase
GPX	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
HGB	haemoglobin
Hr	hour
HSP	heat shock protein
IL	interleukin
IU/L	international unit per liter

L/kg	Liter per kilogram
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MDA	Malon dialdehyde
mg/dL	milligrams per deciliter
mg/ml	milligrams per milliliter
ml	milliliter
mM	millimolar
NAD^+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced)
NaOH	sodium hydroxide
NIBR	national institute for biomedical research
nm	nano meter
NO	nitroxide
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OGTT	Oral Glucose Tolerance Test
OH^-	hydroxide ion
P. falciparum	*Plasmodiun falciparum*
P. malariae	*Plasmodiun malariae*
P. ovale	*Plasmodiun ovale*
P. vivax	*Plasmodiun vivax*
PBS	phosphate buffered saline
PCR	polymerase chain reaction

PCV	packed cell volume
Pfmdr	plasmodium falciparum multi-drug resistance protein
PGE	prostaglandin E
pH	Hydrogen power
PLT	platelet
Q-A	Quinine and Artesunate
RBC	red blood cell
RBS/RBG	random blood sugar/random blood glucose
RNA	ribonucleic acid
ROS	reactive oxygen species
Rpm	Rate per minute
SEM	standard error of mean
SOD	superoxide dismutase
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TNF	tumor necrotic factor
Trpm5	Transient receptors potential cation channel subfamily M member 5
v/v	volume by volume
WBC	white blood cell
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Preamble

Malaria is the leading cause of morbidity and mortality in most sub-Saharan Africa and Southeastern Asia. It is a mosquito-borne disease caused by a plasmodium parasite and transmitted by infected female anopheles mosquitoes (Malik and Khalafalla, 2004). Most of the malaria related morbidities and mortalities are caused by *Plasmodium falciparum* (Soniran *et al*., 2012). Treatment involves the use of anti-malaria agents and supportive measures. In recent times Artemisinin derivatives have given rise to a renewed hope in fight against malaria, although resistance has been reported in places where their mono-therapy is common (WHO, 2009). Combination therapies have been recommended for the treatment of malaria to prevent early development of resistance to anti-malaria agents.

The Artemisinin based combination therapy (ACT), combining any of the Artemisinin derivatives with a longer acting anti-malaria agent in combating malaria, has brought about improved results in alleviating malaria (WHO, 2009).

Artesunate, a semi-synthetic derivative of Artemisinin used in the treatment of malaria when co-administered with Quinine (effective and longer acting anti-malaria) has shown greater efficacy than when either is used alone (Bartoloni *et al.,* 2010). Co-administration of anti-malaria drugs with other supportive drugs like analgesics in patients with malaria to reduce pain and pyrexia may lead to drug-drug interaction resulting to synergistic

effects, changes in toxicity levels of either drug, changes in efficacy of the drugs etc (Bartoloni *et al.,* 2010).

1.2 Statement of Research Problem

Malaria has been a major health problem in much of the tropics and subtropics. It presents a major disease hazard for travelers that go to warmer climates where malaria is endemic. Severe malaria is a medical emergency that requires adequate and prompt treatment, because it progresses rapidly leading to death (Trampuz *et al.*, 2003). The mortality of untreated severe malaria can be 100%, but with anti-malarial treatment, the overall mortality falls to 15-20% (WHO, 2006). Malaria infection mostly affects children under the age of five and pregnant women. It poses substantial risks to the mother, her foetus and the neonate during pregnancy (Stekette *et al*., 2001).

Malaria is associated with many symptoms and complications ranging from that of the uncomplicated malaria to severe malaria symptoms. Hypoglycaemia remains a common and important complication in severe *Plasmodium falciparum* malaria which occurs throughout the clinical course particularly in children and pregnant women (Ogetii *et al.,* 2010). Hypoglycaemia is an independent risk factor for death in severe malaria and a well recognized adverse effect of parenteral quinine (Ogetii *et al.*, 2010). Hypoglycaemia in children is independently associated with poor outcome and an increased mortality predominantly when accompanied by acidaemia or hyperlactataemia. Untreated hypoglycaemia can cause neurological damage and death, since glucose is the brain's main fuel. Hence, treatment with quinine alone can pose health risk on the patient even

though it is effective (Okitolonda *et al*, 1987). Co-administration of quinine with another anti-malaria may be more beneficial.

Quinine and Artesunate combination has been reported to have a synergistic anti-malaria effect (Bartoloni *et al.*, 2010). It is therefore important to investigate the effect of this combination as drug-drug interaction can occur. Anti-malarial drugs are co-administered with NSAIDs and other analgesics and anti-pyretic agents to reduce pain and pyrexia, associated with malaria. Concomitant administration of these drugs can cause change in one or more safety and efficacy outcomes of either of the drug that interacts with it. Increase in polypharmacy, where patients may take many drugs in the course of a day has increased interest in drug-drug interaction.

1.3 Justification

In some areas of the world, mosquitoes carrying malaria parasites have developed resistance to insecticides. In addition, the parasites have developed resistance to some anti-malaria drugs. These conditions have led to difficulty in controlling both the rate of infection and spread of the disease.

There is need for combination therapy in malaria, since monotherapy rapidly leads to drug resistance. Quinine and Artesunate have been combined in some studies and have shown synergistic effect. However, the effect of the combination on some biochemical parameters of the drugs in the body needs to be considered. Artemisinin based combination therapy (ACT) in the treatment of severe and uncomplicated malaria is

currently the front line drug regimen, hence combination with quinine having a longer half life may be considered more beneficial (Trevor *et al.,* 2010). Most drug combinations can result in drug-drug interaction either synergistic or antagonistic side effects which are not supposed to be overlooked (Wong and Townley, 2011). More education is therefore needed by both the patients and the health care giver on the risks and potential interactions of drug preparations and administration. Co-administration of quinine with Artesunate may also limit toxicity. Therefore possible interaction can occur when quinine's combination with Artesunate is co-administered with analgesics and these will need further research and pharmacovigilance (Achan *et al.,* 2011).

Non-steroidal anti inflammatory drugs (NSAIDs) are one of the largest classes of medication in demand worldwide. They are mostly used for inflammation, pain and pyrexia. Most of their uses have been associated with toxicity especially to some body systems like the renal, hepatic, gastro-intestinal, dermatological and central nervous systems (Gupta *et al.,* 2008). Combination of drugs with different mechanisms of action and potency of analgesia can be maximized, while the incidence of adverse effects is minimized (Miranda *et al.,* 2007). Anti-malarial drugs are usually given alongside analgesics although, quinine and Artesunate have been shown to have analgesic, anti-inflammatory and anti-pyretic effects (Achan *et al*., 2011; Mannikoth *et al*., 2012). However, administration of these drugs together with NSAIDs could have additive effects.

1.4 Aim

The aim of this study is to evaluate the effect of Quinine-Artesunate co-administration on glucose level and some other biochemical parameters in Swiss albino mice and rats.

The specific objectives of this study are:

1. To investigate the effect of Quinine-Artesunate co-administration on blood glucose level in healthy and plasmodium parasitized albino mice.
2. To investigate the possible adverse effect of Quinine-Artesunate co-administration on liver function and oxidative stress biomarkers of healthy and plasmodium parasitized albino mice liver homogenates.
3. To investigate the effect of Quinine-Artesunate co-administration on haematological; and on the organ body weight ratio of the liver, spleen and kidney of healthy albino mice.
4. To investigate the effect of Quinine-Artesunate co-administration on the analgesic and antipyretic action of Ibuprofen in healthy albino mice and rats respectively.

1.5 Hypothesis

Quinine-Artesunate co-administration does not have effect on glucose level and some other biochemical parameters in *Plasmodium berghei* parasitized Swiss albino mice and rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria

Malaria is a mosquito borne parasitic disease transmitted by plasmodium. There are four species of this parasite responsible for the human malaria. The most serious and lethal strain of the parasites is *Plasmodium falciparum,* which affects more red blood cells than the other types; *P. vivax, P. malariae and P. ovale* which cause less severe of the infection. Recently, a fifth species of the parasite *Plasmodium knowlesi,* is also responsible for causing malaria in Malaysia and areas of Southeast Asia (Barber *et al*., 2013). These parasites are carried by anopheles mosquito vectors. The anopheles mosquito vectors responsible for transmitting the parasite are of different species, *Anopheles arabiensis*, *Anopheles gambiae* and *Anopheles funestus* (Malik and Khalafalla, 2004).

2.2 Prevalence of Malaria

Malaria is prevalent in tropical and subtropical regions because rainfall, warm temperatures, and stagnant waters provide habitats ideal for mosquito larvae. As earlier stated, malaria remains the predominant cause of illness and death in sub-Sahara African and Southeast Asian countries. High prevalence of malaria is also known in Nigeria (Soniran *et al.,* 2012). Recently, Nigeria was listed by WHO among high burden countries with limited evidence of decrease in malaria cases (WHO, 2010). Children under the age of 5 (five) and pregnant women are mostly at risk of having malaria,

although, it affects all age groups. The economic burden posed to Africa is about 12 billion US dollars per year. In 2011, there were 243 million cases worldwide with at least 655,000 deaths, 85% of which are under the age of five (Noble, 2013). Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world. Malaria is a risk for 97% of Nigerian population with the remaining 3% of which living in malaria free highlands. In Nigeria children under the age of 5 including infants are 56.9% at risk of malaria. There are estimated 100 million malaria cases with over 300, 000 deaths per year in Nigeria. This compares with 215, 000 deaths per year in from HIV/AIDS. Malaria contributes to an estimated 11% of maternal death (WHO, 2011).

2.3 Transmission

Plasmodium parasites are usually transmitted after the bite of an infected female *Anopheles* mosquito. Although infections also occur through transfusion of infected blood (transfusion malaria) and by congenital transmission from mother to her unborn child (congenital malaria), particularly in areas of stable malaria infection whereas, the mosquito is the definitive host and vector (Zucker, 1996). About 20 different species of *Anopheles* are locally important in the transmission of malaria around the world (WHO, 2013).

Malaria can be carried by mosquitoes in temperate climates, but the parasites disappear over the winter. Malaria commonly infects international travelers, sometimes fatally. Disease transmission can also be reduced by preventing mosquito bites by using

mosquito nets and insect repellents, or with mosquito-control measures such as spraying insecticides and draining standing water (WHO, 2013).

2.4 Signs and Symptoms of Malaria

Most of the symptoms of malaria are caused by the release of merozoites into the blood stream. For example, anaemia which results from the destruction of red blood cells; and large amount of free haemoglobin being released into circulation after the breakup of red blood cells.

The clinical signs and symptoms of malaria include, fever and flu-like illness, such as chills, headache, muscle aches and tiredness which may be accompanied by nausea, vomiting and diarrhea. Malaria can also cause anemia because of the loss of erythrocytes and hypoglycaemia especially in children and pregnant women. If not promptly treated, malaria may cause kidney failure, seizures, mental confusion, coma and death (de Souza *et al*., 2010; Nadjm and Behrens, 2012).

2.5 Classification of Malaria

Malaria is classified into either uncomplicated or complicated (severe) malaria by the world health organization (WHO, 2006). It is diagnosed as severe when any of these criteria are present; decreased consciousness, significant weakness such that the person is unable to walk and lack of appetite. Two or more convulsions, low blood pressure, breathing difficulties and circulatory shock are usually present in complicated malaria. Kidney failure or haemoglobin in the urine, bleeding problems, or haemoglobin less than

5 g/dl, pulmonary oedema, hypoglycaemia, acidosis or lactate levels of greater than 5 mmol/l, a parasite level in the blood of greater than 100,000 per microlitre (µl) in low-intensity transmission areas, or 250,000 per µl in high-intensity transmission areas are also present in complicated malaria. In the absence of any of the above criteria, malaria is said to be uncomplicated. Malaria can also be classified as cerebral malaria. According to the WHO, cerebral malaria is defined as a severe *P. falciparum*-malaria presenting neurological symptoms, including coma (with a Glasgow coma scale rating of greater than 11, or a Blantyre coma scale greater than 3), or with a coma that lasts longer than 30 minutes after a seizure (de Souza *et al*., 2010; Nadjm and Behrens, 2012).

2.6 Diagnosis of Malaria

Clinical diagnosis of malaria is usually made on patients having a history of fever for the last 48 hours and lives or has travelled to malaria-endemic areas within the last 30 days. This diagnosis entails making a clinical assessment by following the accurate history of the illness and performing a physical examination (FMOH, 2012). Malaria diagnosis is typically followed by the microscopic examination of thin or thick blood films, Malaria blood smears taken at 6 to 12 hours intervals confirm the diagnosis, or with antigen-based rapid diagnostic tests (RDT). Modern techniques that use the polymerase chain reaction (PCR) to detect the parasite's DNA have also been developed, but these are not widely used in malaria-endemic areas due to their cost and complexity. In diagnosis, during a physical examination, enlarged liver or enlarged spleen may be found (Sanchez *et al*., 2004).

2.7 Life cycle of Malaria Parasite

In a complete cycle, both the human host and the female anopheles mosquito are required by the plasmodium. There are two stages in the life cycle of malaria infection, the asexual and the sexual phase. After a bite of man by the infected female anopheles mosquito, it transmits sporozoites from its salivary glands which rapidly enter the circulation and localize via specific recognition events in liver cells, where they transform, multiply, and develop into tissue schizonts. This liver stage is a primary asymptomatic (pre-erythrocytic or exo-erythrocytic) stage of infection and lasts for about 5 to 15 days, depending on the *Plasmodium* species. One sporozoite develops into a tissue schizont then rupture, each releasing ~20,000 merozoites. These enter the circulation, invade erythrocytes, and initiate the erythrocytic cycle. In erythrocytes, most parasites undergo asexual development from young ring forms to trophozoites and finally to mature schizonts. Schizont-containing erythrocytes rupture, each releasing 6 to 32 merozoites depending on the *Plasmodium* species. These erythrocytic parasites are turned into gametocytes (Miller *et al.,* 2002; Sibley, 2004).

2.7.1 The Asexual Erythrocytic Phase in Humans

Parasites mature within erythrocytes in 48-72 hours. Merozoites released invade more erythrocytes to continue the cycle, which proceeds until death of the host or modulation by drugs or acquired partial immunity. Once plasmodia enter the erythrocytic cycle, they cannot reinvade the liver; thus, there is no tissue stage of infection for malaria contracted by transfusion. It is this process that produces febrile clinical attacks. The merozoites

invade more erythrocytes. For erythrocyte invasion, merozoites bind to specific ligands on the red cell surface (Miller *et al.*, 2002; Sibley, 2004). The periodicity of parasitemia and febrile clinical manifestations depends on the timing of schizogony of a generation of erythrocytic parasites.

2.7.2 The Sexual Stage in Mosquito

When a mosquito bites an infected human the gametocytes from the erythrocytic parasites are transferred to the mosquito. Rapid fertilization occurs in the mosquito. Within 24 hours the zygotes turn into ookinetes and penetrate the gut to form oocysts which later develop into sporozoites. These sporozoites are transferred to the humans after mosquito bite, and the cycle continues.

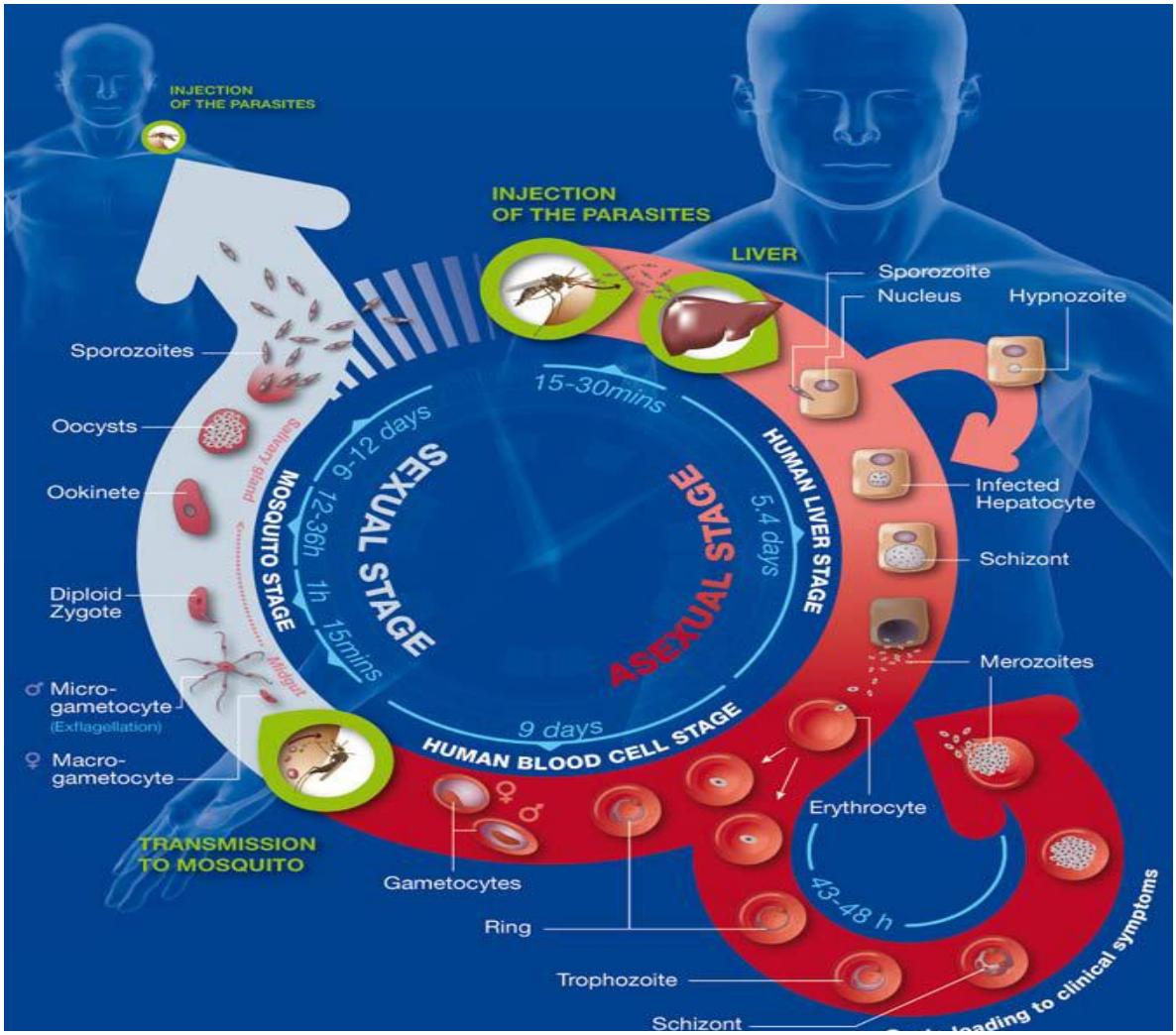


Figure 2.1: Pictorial Representation of Malaria Parasite Life Cycle

(Source: Christian Noble, 2013 Infectious diseases, *NIBR***)**

2.8 Malaria and Glucose Metabolism

The major pathway for carbohydrate metabolism in plasmodia is glycolysis. The pentose phosphate pathway participates in a minor proportion while no activity was found in some plasmodia species such as the *P. vivax*. It has been observed that infected erythrocytes consume more glucose than the uninfected ones (Bass and John, 1912).

Most of the anti-malarials act by inhibiting the enzymes necessary for carbohydrate metabolism and transport in the parasites (Romanha, 2010).

Severe malaria is associated with hypoglycaemia. It occurs both at presentation to hospital and during the course of admission in children with severe malaria. The aetiology of hypoglycaemia is incompletely understood and is likely to be multi-factorial. Depletion of glucose stores due to starvation, parasite utilization of glucose, cytokine-induced impairment of gluconeogenesis have been implicated in malaria (Roe and Pasvol, 2009).

Hyperinsulinaemia, secondary to quinine therapy, has been advanced as an iatrogenic cause and is well established in adults. Data on its relationship in African children with severe malaria are relatively few, but what does exist indicates that hyper-insulinaemia rarely accompanies hypoglycaemia either at admission or during quinine treatment. However, considerable concern still exists and intravenous dosing is strongly recommended in solutions containing dextrose to avert the risk of hypoglycaemia (Ogetti *et al*., 2010).

2.9 Oxidative Stress in Malaria

Oxidative stress is a condition in which cellular antioxidant defenses are inadequate to completely inactivate the reactive oxygen species (ROS) generated. Endogenous antioxidant defenses include glutathione (GSH), antioxidant vitamins and enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and

glutathione peroxidase (GPx), as well as proteins involved in inducible cellular defense mechanisms such as heat shock proteins (HSP). When ROS generation exceeds the rate at which endogenous antioxidants can scavenge it, or when endogenous antioxidant defenses are reduced or weakened, macromolecules become targets for oxidative modifications (Parcario *et al*., 2012). Oxidative damage can severely compromise cell homeostasis and viability, or induce a variety of cellular responses through generation of secondary reactive species, ultimately leading to cell death. The effects of oxidative stress can be gleaned from the analysis of biomarkers of oxidative stress/damage, including oxidatively modified DNA bases, lipid peroxidation end-products, and oxidized proteins (Dalle-Donne *et al*., 2006).

Free radicals through oxidative stress have been involved in the physiopathogenesis of malaria. This involvement of free radicals may be as a result of the host cells' defenses against the parasite or may be triggered by the parasites through pathogenic mechanisms (Parcario *et al*., 2012). The main reason probably for the induction of free radicals and apoptosis in malaria is the generation of hydroxyl radicals (OH^-) in the liver (Parcario *et al*., 2012).

2.10 Treatment of Malaria

It has been recommended by the WHO, that all persons of all ages in all epidemiological settings with suspected malaria should receive a parasitological confirmation of diagnosis by either microscopy or rapid diagnostic test (RDT), and that uncomplicated *P. falciparum* malaria should be treated with Artemisinin-based Combination Therapy

(ACT) (WHO, 2011). ACT has been adopted as national policy for first-line treatment in 79 of 88 countries where *P. falciparum* is endemic (FMOH, 2012).

Malaria, especially severe falciparum malaria, is a medical emergency that requires a hospital stay. The choice of medication depends in part on where one is and when one is infected. Treatment of malaria also depends on severity of the disease. Uncomplicated malaria can be treated with oral medications. The most effective strategy for *P. falciparum* infection is the use of Artemisinins in combination with other anti-malarials, this is known as Artemisinin based-combination therapy (ACT) so as to reduce the ability of the parasite to develop resistance to monotherapy (WHO, 2012).

Chloroquine was often used as an anti-malarial medication. However, chloroquine-resistant infections are common in some parts of the world. Other possible treatments for chloroquine-resistant infections include:

i. The combination of quinidine or quinine plus doxycycline, tetracycline, or clindamycin
ii. Atovaquone plus proguanil (Malarone)
iii. Mefloquine
iv. The combination of pyrimethamine and sulfadoxine (Fansidar)

For severe falciparum malaria, the following drugs are used;

i. Quinine-based regimens
ii. Artemisinin-based regimens
iii. Doxycycline, clindamycin

Medical care, including intravenous fluids, blood transfusions, well equipped laboratory, and other medications such as antibiotics, anti-pyretics, anticonvulsants and respiratory support may be needed during treatment (WHO, 2010).

Anti-malarial drugs can be classified thus;

A. Therapeutic Classification (According to the action in life cycle of malaria parasite)
    a. Causal prophylaxis (Tissue schizontocides)
        i. Proguanil
        ii. Pyrimethamine
        iii. Primaquine (antibiotic)
        iv. Tetracycline (antibiotic)
        v. Fansidar
    b. Clinical cure (Blood schizontocides (erythrocytic cycle))
        i. Chloroquine
        ii. Quinine
        iii. Mefloquine
        iv. Fansidar
        v. Halofantrine
        vi. Tetracycline
        vii. Artemisinin
    c. Gametocides — Primaquine
    d. Hypnozoitocides — Primaquine
    e. Sporozoitocides
        i. Primaquine
        ii. Pyrimethamine

iii. Proguanil

B. Chemical Classification (According to the chemical structure)

i. 4-aminoquinolines; chloroquine, Amodiaquine
ii. Aryl-amino alcohols; Quinine, Mefloquine, Halofantrine
iii. 8-aminoquinolines; Primaquine
iv. Antifolate; Sulfadoxine, Proguanil, Pyrimethamine
v. Antibiotics; Tetracycline
vi. Qinghaosu; Artemisinin
vii. New drugs; Atovaquona, Pyronaridine, Benflumetol (Bruce-Chwatt, 1962)

2.11 Quinine

Quinine is a cinchona alkaloid, which was originally isolated from *Cinchona succirubra.* It is a white crystalline alkaloid that is almost insoluble in water but soluble in alcohol, ether, carbon disulfide, chloroform and glycerol. It is used chiefly (usually in the form of soluble hydrochloride or sulfate salts) in the treatment of falciparum malaria resistant to other anti-malarial drugs. Quinine is preferred where the disease has become highly resistant to other anti-malarial drugs such as quinacrine, chloroquine and primaquine. These other drugs were known to be more effective. In addition to its anti-malarial activity, quinine shows anti-bacteria, antipyretic, mild oxytocic, local anesthetic, cardiovascular stimulant and analgesic properties (Achan *et al*., 2011). It decreases the excitability of the motor endplate. Quinine is used to prevent cardiac arrhythmias and is

used in tonic beverages which are mixed with alcohols for bitter taste. A quinine supplement in diet is known to cause decrease in body weight and food intake in rats. It is also an *in vitro* inhibitor of Transient receptors potential cation channel subfamily M member 5 (Trpm5), a cation channel expressed in taste bud cells, the gastrointestinal tract, pancreas and glucose homeostasis (Cettour-Rose *et al.,* 2013).

2.11.1 Chemistry

Quinine and quinidine are the most important of the Cinchona extracts, which contain a mixture of more than 20 structurally related alkaloids. Both compounds contain a quinoline group attached through a secondary alcohol linkage to a quinuclidine ring. A methoxy side chain is attached to the quinoline ring and a vinyl to the quinuclidine. They differ only in the steric configuration at two of the three asymmetrical centers: the carbon bearing the secondary alcohol group and at the quinuclidine junction. Although quinine and quinidine have been synthesized, they are still obtained from natural sources (Krishna and White, 1996).

2.11.2 Pharmacokinetics

Quinine is readily absorbed when given orally or intramuscularly. In oral administration, absorption occurs mainly from the upper small intestine and is more than 80% complete, even in patients with marked diarrhea. After a single oral dose, plasma levels of quinine reach a maximum in 3 to 8 hours and, after distributing into an apparent volume of about 1.5 L/kg in healthy individuals, declines with a half-life of about 11 hours after termination of therapy. As reviewed by Krishna and White (1996), the pharmacokinetics

of quinine may change according to the severity of malarial infection. Values for both the apparent volume of distribution and the systemic clearance of quinine decrease, the latter more than the former, so that the average elimination half-life increases from 11 to 18 hours. After standard therapeutic doses, peak plasma levels of quinine may reach 15 to 20 mg/L in severely ill patients without causing major toxicity; in contrast, levels greater than 10 mg/L produce severe drug reactions in self-poisoning. The high levels of plasma α_1-acid glycoprotein produced in severe malaria may prevent toxicity by binding the drug and thereby reducing the free fraction of quinine. Concentrations of quinine are lower in erythrocytes (33% to 40%) and CSF (2% to 5%) than in plasma and the drug readily reaches fetal tissues (Newton *et al*., 1999).

The cinchona alkaloids are metabolized extensively, especially by hepatic CYP3A4 (Zhao *et al.,* 1996), so only about 20% of an administered dose is excreted unaltered in the urine. There is no accumulation of the drugs in the body on continued administration. However, the major metabolite of quinine, 3-hydroxyquinine, retains some anti-malarial activity and can accumulate and possibly cause toxicity in patients with renal failure (Newton *et al.,* 1999). Renal excretion of quinine itself is more rapid when the urine is acidic.

2.11.3 The Mechanism of Action of Quinine

It complexes with double strand DNA to prevent strand separation which results in blockade of DNA replication and transcription to RNA. This is thought to inhibit nucleic acid synthesis, protein synthesis and glycolysis in *Plasmodium falciparum* and bind with

haemazoin in parasitized erythrocytes. Quinine is solely a blood schizontocide (Newton *et al*., 1999).

2.11.4 Resistance

Increasing *in vitro* resistance of parasites from a number of areas suggests that quinine resistance was an increasing problem. Resistance to quinine is already common in some parts of Southeast Asia, especially border areas of Thailand, where the drug may fail if used alone to treat falciparum malaria. However, quinine still provides at least a partial therapeutic effect in most patients. The basis of *P. falciparum* resistance to quinine is complex. Patterns of *P. falciparum* resistance to quinine more closely resemble those of resistance to mefloquine and halofantrine rather than to chloroquine. Amplification of *Plasmodium falciparum* multi drug resistant drug (*pfmdr1*) in *P. falciparum*, implicated in resistance to mefloquine and halofantrine, can also confer resistance to quinine *in vitro.* However, the correlation is inconsistent (Dorsey *et al.,* 2001; Sidhu *et al.,* 2002). Quinine and quinidine sensitivity also can change in different strains (Sidhu *et al.,* 2002). Recent evidence suggests the participation of a number of different transporter genes in conferring resistance to quinine (Mu *et al.,* 2003). It should not be used routinely for prophylaxis to delay emergence of resistance (Trevor *et al.,* 2010).

2.11.5 Therapeutic uses

2.11.5.1 Anti-malarial Actions

Quinine is widely used in chemotherapy of malaria especially in chloroquine resistant and cerebral malaria (White and Warrel, 1983; Hall and Peters, 1985). Quinine acts

primarily against asexual erythrocytic forms of malaria parasite and has little effect on hepatic forms of the parasites. It is a rapidly acting, highly effective blood schizonticide against the four species of human malaria parasites. The drug is gametocidal against *P. vivax* and *P. ovale* but not *P. falciparum*. However, quinine, and its stereoisomer quinidine, is especially valuable for the parenteral treatment of severe illness owing to drug-resistant strains of *P. falciparum,* even though these strains have become more resistant to both agents in certain parts of Southeast Asia and South America. Because of its toxicity and short half-life, quinine generally is not used for prophylaxis. It is commonly used with doxycycline or clindamycin to shorten the duration of therapy and limit toxicity level (Trevor, *et al.,* 2010).

2.11.5.2 Action on Skeletal Muscle

Quinine and related cinchona alkaloids exert effects on skeletal muscle. Quinine increases the tension response to a single maximal stimulus delivered to muscle directly or through nerves, but it also increases the refractory period of muscle so that the response to tetanic stimulation is diminished. The excitability of the motor end-plate region decreases so that responses to repetitive nerve stimulation and to acetylcholine are reduced. Thus, quinine can antagonize the actions of physostigmine on skeletal muscle as effectively as curare. Quinine may also produce alarming respiratory distress and dysphagia in patients with myasthenia gravis. Quinine may cause symptomatic relief of myotonia congenita (Trevor *et al*., 2010).

2.11.5.3 Babesiosis and Other Uses of Quinine

Quinine is first-line therapy, in combination with clindamycin, in the treatment of infection with *Babesia microti* or other human babesial infections. Quinine can be used as an abortifacient because of its mild oxytoxic effects. It has also been used as anti-inflammatory, a weak antipyretic and analgesic (Achan *et al*., 2011).

2.11.6 Adverse Effects

Quinine is associated with so many adverse effects including cinchonism, hypersensitivity reactions which include skin rashes, urticaria, angioedema and bronchospasm. Among the side effects is high-frequency hearing loss, impaired vision including blindness, nausea, and vomiting and epigastric pain. Hematologic abnormalities which include hemolysis (especially with G6PD deficiency), leukopenia, agranulocytosis, and thrombocytopenia are also adverse effects associated with quinine. Other adverse effects include granulomatous hepatitis, cardiovascular effects including cardiac conduction abnormalities, vascular instability with postural hypotension. Therapeutic doses may cause hypoglycemia as a consequence of stimulation of insulin release leading to hyperinsulinemia (Elbadawi *et al*., 2011). This is a particular problem in severe infections and in pregnant patients, who have increased sensitivity to insulin (Jaeger *et al.*, 1987). Intravenous infusions of quinine and quinidine may cause thrombophlebitis. Quinine can stimulate uterine contractions, especially in the third trimester. However, this effect is mild, and it remains the drug of choice for severe falciparum malaria even during pregnancy. Severe hypotension can follow intravenous infusions of quinine or quinidine given very rapidly (Cettour-Rose, 2013).

2.11.7 Contraindications and Drug Interactions

Quinine is contraindicated in patients with a history of hypersensitivity and in the presence of haemoglobinuria during malaria and in patients with optic neuritis, or tinnitus (Karlsson *et al.*, 1990). It should be used with caution in patients with atrial fibrillation and other myocardial conduction abnormalities because of its adverse cardiovascular effects (it causes increase in heart rate) (Achan *et al*., 2011). In patients with myasthenia, quinine may cause severe respiratory distress and dysphagia. In patients with glucose-6-phosphate deficiency, black water fever may be observed when quinine is used (Karlsson *et al.*, 1990). It should not be given concurrently with mefloquine and should be used with caution in malarial patients who has previously received mefloquine chemoprophylaxis to prevent cardiotoxicity. Absorption may be blocked by aluminum-containing antacids. Quinine may enhance the effects of anticoagulants; it can raise plasma levels of warfarin and digoxin since cytochrome P450 3A4 has been shown to be important in the metabolism of quinine. Dosage must be reduced in renal insufficiency (Karlsson *et al.*, 1990).

2.12 Artesunate: Artemisinin Derivative

Artemisinin based anti-malarials are drugs with unique structures and mode of action. They have been shown to have faster parasite clearance time and faster relief of symptoms (Sinclair *et al*., 2009). Artemisinin is a sesquiterpene lactone endoperoxide, a natural product derived from the weed; qing hao (*Artemisia annua*) also called sweet wormwood (Woodrow *et al.*, 2005). It is the active component of a herbal medicine that

has been used in China as an anti pyretic for over 2000 years (Klayman, 1985). Three semi-synthetic derivatives with improved potency and bioavailability have since largely replaced the use of artemisinin. These include dihydroartemisinin, a reduced product; artemether, an oil-soluble methyl ether; and artesunate, the water-soluble hemisuccinate ester of dihydroartemisinin. Other derivatives of artemisinin include arteether and artemoil. The Artemisinins are potent and fast-acting anti-malarials. They are particularly well suited for the treatment of severe *P. falciparum* malaria and now play a key role in the combination therapy of drug-resistant infections. Artemisinin and its derivatives are the most important class of anti-malarial drugs effective for both uncomplicated and severe malaria (Maude *et al.,* 2010). Millions of patients, especially in China and Southeast Asia, have been treated with endoperoxides, which generally are regarded to be safe (Newton *et al.*, 2003b).

The mechanism of action of these drugs depends on the alkylation of plasmodium-specific proteins, which prevents the formation of hemozoin and consequently causes the release of free iron that is toxic to the parasite (Kamchonwongpaisan and Meshnick, 1996). However, literature has shown that artesunate also has immunomodulatory effects on mammalian cells (Li *et al.,* 2006).

Apart from the above, Artemisinin and its derivates have been shown to possess anticancer (Efferth *et al.,* 2001), antiviral (Kaptein *et al.,* 2006), and anti-inflammatory (Mirshafiey *et al.,* 2006; Tawfik *et al.,* 1990; Veerasubramanian *et al.,* 2006 and Wang *et al.,* 2006) activities.

Artesunate has been compared with quinine in terms of efficacy and was found to be more superior in the time for clearing both fever and parasite but as good as quinine in terms of preventing mortality, although quinine is more preferable than Artesunate in cerebral malaria because it has a better recovery from coma (Patel *et al*., 2003).

Artesunate has been reported to block the production of IL-1β, IL-6 and IL-8 from TNF-α-stimulated human rheumatoid arthritis fibroblast-like synoviocytes. In addition, it inhibits expression of toll-like receptor 4 induced by heat-killed *E. coli* lipopolysaccharide. It also inhibits production of TNF-α, IL-6 and nitric oxide (NO) (Xu *et al*., 2007). There are also evidences that artesunate attenuates experimental allergic airway inflammation via negative regulation of PI3 K/Akt signaling pathway (Cheng *et al.,* 2011). The effect of artesunate on inflammatory markers was studied during malaria infection and demonstrated that artesunate is able to exert a protective effect against the *P. berghei*-induced inflammatory response by inhibiting NF-κB nuclear translocation and the subsequent expression of ICAM-1 in endothelial cells. The anti-malarial activity of artemisinin and its derivatives has been well demonstrated *in vitro* and *in vivo* (Janse *et al.,* 1994; de Vries and Dien, 1996).The efficacy of anti-malarial drugs in an experimental model was addressed, with findings showing that artemether and artesunate show the highest efficacies in rescuing mice with late-stage cerebral malaria (Clemmer *et al.,* 2011).

2.12.1 Mechanism of Action of Artesunate

Artesunate is extensively hydrolyzed by plasma esterases and perhaps also by CYP2A6. Its main metabolite, dihydro-artemisinin (DHA) is presumed to account for most of the *in vivo* anti-malarial activity. DHA is metabolized through glucuronidation (Xie, 2005).

2.12.2 Combination Therapy

Combination therapy is defined as the simultaneous use of two or more blood schizontocidal drugs with independent modes of action and different biochemical targets in the parasite. The most successful attempts at combating malaria have been in the administration of combination therapy. The combinations of drugs currently prescribed are divided into two categories;

(i) Non-artemisinin and Quinine based combinations and

(ii) Artemisinin based combinations (ACT).

World Health Organization recommends that *P. falciparum* malaria be treated always with a combination of two drugs acting at different biochemical sites within the parasites (WHO, 2006). World Health Organization recommendations list artemether plus lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine, and artesunate plus sulfadoxine plus pyrimethamine as optimal therapies for uncomplicated malaria in regions with resistance to older drugs (WHO, 2012). Artesunate is commonly used in combination with mefloquine to treat highly resistant falciparum malaria in Southeastern Asia and most places Artemether is available alone and as a fixed-dose combination with

lumefantrine. In the setting of multidrug resistance, many authorities now advocate combination therapy with an artemisinin as the optimal treatment for falciparum malaria.

2.13 Ibuprofen

Ibuprofen, a propionic acid is the most commonly used and frequently prescribed Non-Steroidal anti-inflammatory drug (NSAID) with an anti-inflammatory, analgesic and an anti-pyretic activity. It is a non-specific cycloxygenase (COX) inhibitor resulting in decreased prostaglandin formation. Prostaglandins play important roles in production of inflammation, pain and pyrexia. Although, it has a weaker anti inflammatory action, it has a prominent analgesic and anti pyretic action (Burke *et al*., 2006).

Ibuprofen is supplied with a potency of 200-800 mg per caplet. It is usually given 3 times daily. It is well absorbed orally with peak plasma concentrations reached at 1-2 hours following oral administration. It is bio transformed rapidly with a serum half life of 1.8-2 hours. It is more than 99 % protein bound and extensively metabolized in the liver. The majority is excreted in the urine within 24 hours with a small amount also excreted through the stool. Excretion is virtually 100% within 24 hours of the last dose (Bushra and Aslan, 2010).

NSAIDS are widely used, inappropriately taken and potentially dangerous (Wilcox *et al*., 2005). Nevertheless, Ibuprofen exhibits few adverse effects. The major adverse reactions affect the gastro intestinal tract, kidney and coagulation system. Its side effects include Gastro Intestinal tract ulceration, blood thinning effects (avoid use with blood thinning

drugs), decrease in efficacy of blood pressure lowering drugs, and an interference with secretion of lithium and aminoglycosides that can result in increased blood levels of those drugs. Other side effects include thrombocytopenia, headache, dizziness, rashes, blurred vision, fluid retention, oedema and in some cases toxic amblyopia (Rocca *et al*., 2005; Bushra and Aslsam, 2010).

2.14 Drug-Drug Interaction

Drug-drug interaction is a type of interaction in which the pharmacological effects are produced by two or more drugs administered concurrently or consecutively. It can lead to synergistic effect, potentiation , additive effect, or antagonistic effect and it can also lead to increase in toxicity level of the drugs. One of the factors that can alter the response to drugs is the concurrent administration of other drugs. Interest in drug-drug interactions has also increased because of the rise in polypharmacy, where patients have to take many drugs in the course of a day (Bedford *et al.*, 1996). All effective drugs have the potential for producing both beneficial and harmful effects related to the desired and undesired effects. The particular response to a drug by a patient is driven in one way or another by the concentration of that drug and/or its metabolites at the target sites within the body. Usually, individuals vary in their responses to drugs which may be due to genetic differences, age, disease condition and the preference of other drugs which may interfere with the response pattern, polypharmacy etc. (GIDI, 2012).

There are several mechanisms by which drugs may interact, but most can be categorized as pharmacokinetic (absorption, distribution, metabolism, excretion), pharmacodynamic,

or both interactions (Williamson, 2003). Knowledge of the mechanism by which a given drug interaction occurs is often clinically useful, since the mechanism may influence both the time course and the methods of circumventing the interaction. Some important drug interactions occur as a result of two or more of these mechanisms (Blanchard *et al*., 2004).

Pharmacokinetic drug-drug interactions result from alteration in the dose/systemic exposure relationship, as reflected in a blood or plasma concentration–time curve, when an interacting drug induces or inhibits one or more routes of elimination or transport of a substrate drug. Inhibition of metabolism may be associated with increased blood levels and pharmacological activity of the substrate, but if the substrate is a prodrug, pharmacological activity may be reduced; in some cases, when the parent drug and its metabolite have equal effects, there may be no change in pharmacological activity despite large changes in blood levels of parent and metabolite (Bedford *et al.*, 1996).

Pharmacodynamic drug-drug interaction may arise when the substrate and interacting drug affect the same physiological system or when one drug prevents an appropriate response to the other. Both pharmacokinetic and pharmacodynamic drug-drug interactions should be considered when two or more drugs are administered concurrently (Shiew-Mei Huang, 2008).

2.14.1 Quinine-Artesunate Combination

So many studies have compared the anti-plasmodial efficacy of Quinine-Artesunate co-administration with either of the drugs administered alone. It was found that quinine

administration followed by Artesunate yielded a better anti-malaria result (Newton *et al.*, 2001; Newton *et al*., 2003a). The combination of quinine and artesunate has been shown to have a greater anti-malarial effect than when either drug is administered alone (Dondorp *et al.*, 2005; Bartoloni *et al*, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Laboratory Animals

Adult Albino rats of wistar strains and Swiss albino mice were used in this study. The animals were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. They were housed in clean cages with saw dust as beddings containing 5-7 rats or mice and were used according to the animal care guidelines (National Institute of Health). The bedding was replaced every three days. The animals were fed with standard vital feed and given access to water from public supply *ad libitum* till the end of the experiment except otherwise required by specific experimental protocols such as fasting blood glucose determinations. They were allowed to acclimatize to the animal house conditions of temperature and humidity for a period of two days prior to the commencement of the study. All the experimental drugs were administered orally.

Animals used for the experiments were identified using a simple coded identification which was applied using concentrated picric acid solution or a permanent marker which were re-applied whenever fading was observed.

3.2 Chemicals, Drugs, Reagents and other Materials

The dosage forms of the drugs were used for the study. Acetic acid, brewer's yeast, Phosphate buffered saline, Quinine (Embassy, India; batch number 30049063) 60 mg/kg,

Artesunate (Mekophar, Vietnam; batch number 10005FX) 30 mg/kg and Ibuprofen (Hovid, Malaysia; batch number BCO7509) 100 mg/kg. Digital weighing balance, syringes, needles, micro pipettes, cotton wool, digital Accu-chek Advantage glucometer, Accu-check glucometer strips (Roche, U.S.A; LOT number 571911), observation chamber, hand gloves, dissecting kits, stop watch and record book.

3.3 Preparation of Drugs

Artesunate solution was prepared by weighing 30 mg of the Artesunate and dissolving it in 1 ml of normal saline to obtain the working concentration of 30 mg/ml and this was vigorously shaken for proper dissolution. The working concentration of Quinine (60 mg/ml) was prepared by dissolving 300 mg in 5 ml normal saline with vigorous shaking for proper dissolution. Ibuprofen was prepared by dissolving 100 mg in 1 ml of normal saline to obtain 100 mg/ml working concentration.

3.4 Study Design

All treatments were administered orally at the following doses; Normal saline 10 ml/kg, Artesunate 30 mg/kg, Quinine 60 mg/kg and Ibuprofen 100 mg/kg.

Table 3.1: Experimental design of drug administration in the plasmodium parasitized mice

Groups	Treatment	Dose
Group 1	Normal Saline	10 ml/kg
Group2	Normal Saline (Parasitized)	10 ml/kg
Group 3	Quinine	60 mg/kg
Group 4	Artesunate	30 mg/kg
Group 5	Quinine + Artesunate	60-30 mg/kg

Table 3.2: Experimental design of drug administration in the Healthy (uninfected) mice

Groups	Treatment	Dose
Group 1	Normal Saline	10 ml/kg
Group2	Quinine	60 mg/kg
Group 3	Artesunate	30 mg/kg
Group 4	Quinine + Artesunate	60-30 mg/kg

Table 3.3: Experimental design of drug administration in algesic mice and pyretic rats

Groups	Treatment	Dose
Group 1	Normal Saline	10 ml/kg
Group2	Quinine + Ibuprofen	60-100 mg/kg
Group 3	Artesunate + Ibuprofen	30-100 mg/kg
Group 4	Quinine + Artesunate + Ibuprofen	60-30-100 mg/kg
Group 5	Ibuprofen	100 mg/kg

3.5 Malaria Parasitized Animals

The mice were infected with malaria parasite *Plasmodium berghei* (strain NK65) a donor mouse obtained from the Department of Pharmacology and Therapeutics Ahmadu Bello University, Zaria. *P. berghei*-infected red blood cells were obtained from the tail vein of infected mouse and diluted with phosphate buffered saline (PBS) so that each 0.2 mL that was subsequently injected contained approximately 10^3 infected red cells (parasite) per kilogram of body weight. The infection of the recipient mice were initiated by needle passage of the parasite preparation from the donor to healthy test animals via the intraperitoneal route as described by David *et al.*, (2004) and Peter and Anatoli (1998). Fifth day after establishment of infection, drugs were administered once daily for seven days to all the groups per body weights (Table 3.1). Parasitaemia was checked on day 1 and day 7 of treatment following Geimsa staining of thin blood smear.

3.6 Glucose Level on Healthy Animals (Uninfected mice)

Another set of uninfected mice were grouped into four with each group containing 7 mice as shown on Table 3.2. They were grouped according to their body weights having an average of 23 g. The animals were fasted for 16 hours but given access to water *ad libitum*. Their blood glucose level was taken using an Accu-chek glucometer with compatible strips on day 0, day 1 and day 7 post treatment.

3.6.1 Oral Glucose Tolerance Test

Oral glucose tolerance test (OGTT) was performed on the animals on day 7 post treatment as described by Syiem *et al*., (2002). Although OGTT is a test performed on

diabetogenic animals to see the effect of treatment on blood sugar level, it was also carried out in this study to see if it will have effect on the blood glucose level of the animals. The animals were weighed and their basal blood glucose level was taken. They were administered with drugs according to Table 3.2, after an hour glucose solution (2 g/kg) was administered to each mouse and the blood glucose level was taken at 30 minutes, 60 minutes and 120 minutes. On the 8th day the animals were euthanized using chloroform to anaesthesize the animals. The kidney, spleen, liver and brain were removed, weighed and recorded. The blood samples were collected for biochemical and haematological analysis.

3.7 Biochemical Parameters Analysis

The animals were euthanized with chloroform as anaesthesia and blood samples from the jugular vein was collected in sterile labeled plain sample tubes and centrifuged at 3000 rpm for 10 min to obtain a clear serum using a centrifuge (Edewor *et al.*, 2010). The liver of each animal was weighed and homogenized using mortar and pestle in phosphate buffered saline pH 7.2 and for each gram of the liver, 10 ml of the buffer was used. The homogenized samples were centrifuged at 5000 rpm for 30 minutes and the clear homogenates were collected and used for antioxidant and liver function test analysis. Liver homogenates were used for analysis due to insufficient blood sample for liver function test.

3.7.1 Liver Function Tests

Aspartate amino transferase, Alanine amino transferase and Alkaline phosphatase were determined using colorimetric kits of analytical standard grade.

3.7.1.1 Aspartate Amino Transferase (Glutamic-Pyruvic Transaminase, GPT)

In this reaction, L-Alanine and α-ketoglutarate react in the presence of GPT in the sample to yield pyruvate and L-glutamate. Pyruvate is reduced by lactate dehydrogenase to yield lactate with oxidation of NADH to NAD. The reaction was monitored by measurement of the absorbance of NADH at 340 nm. The rate of reduction in absorbance is proportional to GPT activity in the sample.

One milliliter of the working reagent (Reckon Diagnostics) was mixed immediately with 0.05 ml of the sample and the first absorbance of test was read exactly at 1 minute and thereafter at 30, 60, 90 and 120 secs at 340 nm. The mean change in absorbance per minute was determined (ΔA/min) and test results calculated thus:

GPT activity (IU/L) = ΔA/min. x F. Where F = 3376 (based on the millimolar extinction coefficient of NADH at 340 nm)

3.7.1.2 Aspartate Amino Transferase (Glutamic Oxaloacetic Transaminase, GOT)

In this reaction, L-Aspartate and α-Ketoglutarate react in the presence of GOT in the sample to yield oxaloacetate and L-glutamate. The oxaloacetate is reduced by malate dehydrogenase (MDH) to yield L-malate with the oxidation of NADH to NAD. The reaction was monitored by measurement of the decrease in absorbance of NADH at 340 nm. The rate of deduction in absorbance is proportional to GOT activity in the sample.

One milliliter of the working reagent (Reckon Diagnostics) was mixed immediately with 0.05 ml of the sample and the first absorbance of test was read exactly at 1 minute and thereafter at 30, 60, 90 and 120 secs at 340 nm. The mean change in absorbance per minute was determined (ΔA/min) and test results calculated thus:

GOT activity (IU/L) = ΔA/min. x F. Where F = 3376 (based on the millimolar extinction coefficient of NADH at 340 nm)

3.7.2 Oxidative Stress Biomarkers

Thiobarbituric acid reactive substances (TBARS) assay. The thiobarbituric acid (TBA) assay was used to assess lipid peroxidation using the method of Bar-Or *et al*., (2001), with a few modifications. All fatty acids were initially prepared as stock solutions in acetone to facilitate solvation. In the final reaction mixture, individual fatty acid concentrations ranged from 0-0.2 mM, while the acetone concentration was 0.5% (v/v). Samples were pre-incubated for 15 min with a fatty acid or 0.5% acetone (v/v; positive control) and 0.01 mM $CuCl_2$ in 20 mM phosphate buffer (pH 7.4). The reaction was started by the addition of 0.5 mM ascorbate and 1.9 mM deoxyribose, and incubated for 1 hour at 37°C. 10 g/L TBA in 50 mM NaOH and concentrated acetic acid were added to the incubation mixture, boiled in water for 15 min and the thiobarbituric acid-reactive species (TBARS) quantified by spectrophotometry at 532 nm. The TBARS in the fatty acid containing samples were compared with the positive controls containing 0.5% (v/v) acetone.

Oxidative stress enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) were measured using Northwest life science specialties activity assay kit. The samples were prepared according to the manufacturers guidelines and read spectrophotometrically at 240 nm after every 2 seconds for 15 seconds for catalase; 560 nm every 10 seconds for at least 5 minutes for SOD and 340 nm for every 30 seconds for 5 minutes for GPX.

3.8 Haematological Parameters Analysis

From each animal, blood samples were collected from the jugular vein into clean labeled EDTA sample tubes. Packed cell volume, red blood cell, white blood cell, platelet count, haemoglobin, mean cell volume, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin were determined using the Sysmsex Automated Haematology Analyzer KX-2IN, Sysmsex Corporation, Kobe-Japan at Ahmadu Bello University Teaching Hospital, Shika and values were taken.

3.9 Organ Body Weight Ratio

The kidney, spleen and the liver were removed from the animals in all groups on the eight day of the experiment after being sacrificed and weighed. The relative organ body weights were determined thus, $\frac{\text{weight of organ (g)}}{\text{weight of animal on sacrifice day (g)}}$ X 100

3.10 Analgesic Study: Acetic Acid Induced Abdominal Constrictions

Writhing was induced in mice as described by Koster *et al.,* 1959. The mice received drugs (orally) according to their body weight in their respective groups. Group 1 was

administered Normal saline (10 ml/kg), Group 2 was administered Quinine (60 mg/kg), Group 3 was administered Artesunate (30 mg/kg) and Group 4 was administered Quinine and Artesunate (60 and 30 mg/kg). Thirty minutes after, the mice received 0.6 % acetic acid (10 ml/kg, intraperitoneally) to induce pain (Kumar *et al.,* 2012). The acetic acid injected animals were placed in an observational box (Hong *et al.,* 2011). Five minutes after acetic acid injection, the number of abdominal writhes by each mouse were counted for 20 minutes and recorded. Percentage Pain Inhibition (%) was determined thus,

$$\frac{\text{Mean number of writhes (normal control)-Mean number of writhes (test)}}{\text{Mean number of writhes (normal control)}} X\ 100$$

3.11 Anti-Pyretic Study: Brewer's Yeast Induced Pyrexia Model

Antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rats as described by Kumar *et al.,* 2009. Fever was induced by administering 20 ml/kg of 20% aqueous suspension of Brewer's yeast in normal saline subcutaneously. Nineteen hours after the injection of the yeast, the rectal temperature was taken and immediately administration of the drugs according to the body weights of the animals was carried out. The rectal temperature was determined using a digital thermometer at 1, 2, 3, and 4 hours post treatment. Drugs were administered according to table 3.1.

3.12 Statistical Analysis

Results were expressed as mean $\pm$ standard error of mean. One way analysis of variance (ANOVA) followed by homogeneity of variances and a suitable post hoc test was chosen, repeated measure ANOVA and Bonferroni Post Hoc test were used depending on data

type to analyze and compare the results at a 95% confidence level using SPSS version 20 and Microsoft excel.

CHAPTER FOUR

RESULTS

4.1 The Effect of Quinine, Artesunate, Quinine-Artesunate Co-administration and their Separate Therapy on Blood Glucose Level in Swiss Albino Mice

Quinine-Artesunate co-administration on Fasting Blood Glucose levels in healthy animals did not show significant difference within the groups when compared across the days (Table 4.1). A statistical insignificant reduction in Blood Glucose level was seen in quinine group on days 0, 1 and 7 compared to the normal control group. Quinine-Artesunate treated group showed insignificant reduction when compared to the control group on the 7th day.

Table 4.1: The Effect of Quinine-Artesunate Co-administration on Fasting Blood Glucose Level in Swiss Albino Mice

Group	Mean Values± SEM of Fasting Blood Glucose Level (mg/dL)					
	FBS Day 0	**% Reduction**	**FBS Day 1**	**% Reduction**	**FBS Day 7**	**% Reduction**
Normal control	99.29 ±5.59	0	116.43 ±8.32	0	100.71 ±11.35	0
Quinine	92.86 ±8.04	6.48	99.14±6.61	14.85	86.57 ±11.09	14.04
Artesunate	118.71 ±12.37	-19.56	95.00 ±11.62	18.41	96.14 ±13.26	4.54
Quinine+ Artesunate	96.60 ±9.64	2.71	104.60 ±5.15	10.16	83.00 ±11.13	17.59

N=7, FBS: Fasting blood sugar, SEM: standard error of mean
Repeated measures ANOVA and Bonferroni Post Hoc test

4.2 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Oral Glucose Tolerance Test in Swiss Albino Mice

Results were compared between and within the groups across the times as well as between treatment groups. Quinine-Artesunate co-administration did not show significant difference across the time. Results showed significant increase ($P<0.01$) in blood glucose levels between time 0 and 30 minutes in all the groups except the Quinine-Artesunate group. In the normal control group, a significant decrease was seen when the time 30 minutes was compared to time 60 minutes ($P<0.05$) and 120 minutes ($P<0.01$). A significant decrease was seen when time 60 minutes was compared to time 120 minutes ($P<0.05$). In Quinine group, a significant decrease was found when time 30 minutes was compared 60 minutes ($P<0.05$) and 120 minutes ($P<0.001$). In Artesunate group, a significant decrease was found when time 30 minutes was compared to time 60 minutes ($P<0.05$) and when time 60 minutes was compared to time 120 minutes ($P<0.05$) (Table 4.2). The results did not show any significant difference between the groups.

Table 4.2: The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Oral Glucose Tolerance Test of Swiss Albino Mice

Group	Mean Values ± SEM of Oral Glucose Tolerance Test (mg/dL)						
	Basal	**30 min**	**% Elevation**	**60 min**	**% Reduction**	**120 min**	**% Reduction**
Normal control	100.71 ±11.34$^{\#\#}$	319.14 ±35.36**	216.89	164.14 ±17.12*$^{\#}$	48.57	85.14 ±4.75$^{\#\#a}$	48.13
Quinine	86.57 ±11.09$^{\#\#}$	228.43 ±17.74**	164.00	138.43 ±17.81$^{\#}$	39.39	90.43 ±9.55$^{\#\#\#}$	34.67
Artesunate	96.14 ±13.26$^{\#}$	274.71 ±43.51*	185.73	152.29 ±12.15	44.56	90.57 ±9.24$^{\#a}$	40.53
Quinine+ Artesunate	93.00 ±13.51	236.33 ±51.61	154.12	118.50 ±21.63	49.86	87.33 ±10.69	26.30

N=7, Repeated measures ANOVA and Bonferroni Post Hoc test, SEM: standard error of mean; *=P<0.05, **=P<0.01, vs. time 0; $^{\#=}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#=}P$<0.001 vs. time 30; ^{a} = P<0.05 vs. time 60

4.3 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Liver Function Test of Albino Mice

A significant increase in AST level of Quinine group compared to Artesunate group was found ($P<0.05$). There was no significant difference when the co-administration was compared to the normal control, quinine and artesunate groups. There was an insignificant increase in the ALP of quinine compared to the control and other treated groups. (Table 4.3)

Table 4.3: The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Liver Function Test in Swiss albino mice

Group	Mean Values ± SEM of Liver Biomarkers (IU/L)		
	ALT	**AST**	**ALP**
Control	1596.43 ±78.91	1497.29 ±35.76	1486.00 ±90.44
Quinine	1591.29 ±61.66	1652.86 ±48.38*	1613.29 ±73.26
Artesunate	1563.43 ±68.10	1446.14 ±60.28	1536.57 ±62.23
Quinine + Artesunate	1505.50 ±59.15	1558.50 ±54.66	1592.00 ±52.23

N=7, ALT, Alanine amino transferase; ALP, Alkaline phosphatase; AST, Aspartate amino transferase; *=$P<0.05$ vs. Artesunate group; ANOVA and Bonferroni Post Hoc test

4.4 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Oxidative Stress Biomarkers on Swiss Albino Mice

Oxidative stress biomarkers of the Quinine-Artesunate co-administration and other groups did not show any significant difference when compared between the groups. There was an insignificant increase in SOD and CAT of Artesunate group compared to other groups (Table 4.4).

Table 4.4: The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Oxidative Stress biomarkers Test in Swiss Albino Mice

Group	Mean Values ± SEM of Oxidative Stress Biomarkers (IU/L)			
	SOD	MDA	CAT	GPX
Control	1.88 ±0.27	2.03 ±0.28	40.83 ±4.04	43.33 ±3.47
Quinine	1.94 ±0.18	1.50 ±0.13	42.29 ±2.71	41.14 ±1.78
Artesunate	2.09 ±0.14	1.66 ±0.14	45.71 ±3.77	37.86 ±2.09
Quinine+Artesunate	1.57 ±0.11	2.08 ±0.11	38.83 ±2.44	35.83 ±1.64

N=7, MDA**,** Malondialdehyde; SOD, Superoxide dismutase; GPX, Glutathione peroxidase; CAT, Catalase; SEM: standard error of mean; ANOVA and Bonferroni Post Hoc test

4.5 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Hematological Parameters in Swiss Albino Mice

A significant increase was seen in the Mean Corpuscular Haemoglobin Concentration level of Artesunate group ($P<0.05$) when compared to the normal control group. There was no significant difference when the co-administration was compared to the normal control, quinine and artesunate groups (Table 4.5).

Table 4.5: The effect of Quinine-Artesunate Co-administration and their Separate Therapy on Haematological Indices in Swiss albino mice

Hematological Indices	Normal Control	Quinine	Artesunate	Quinine-Artesunate
RBC (10^{12}/L)	9.05 ±0.29	8.98 ±0.56	8.68 ±0.57	8.02 ±0.49
PCV (%)	53.39 ±1.63	51.41 ±3.29	45.60 ±3.06	45.13 ±2.52
HGB (g/dL)	15.16 ±0.46	14.84 ±0.86	14.14 ±0.95	13.82 ±0.75
MCH (pg)	16.76 ±0.37	16.79 ±.25	16.30 ±0.31	17.25 ±0.25
MCHC (g/dL)	28.69 ±0.45	29.30 ±0.58	31.08 ±0.69*	30.65 ±0.26
MCV (fL)	58.56 ±1.78	57.49 ±1.74	52.60 ±1.34	56.40 ±0.88
WBC (10^9/L)	5.74 ±1.03	5.93 ±0.97	7.62 ±1.49	8.75 ±2.50
PLT (10^9/L)	598.14 ±103.76	572.00 ±82.63	675.20 ±65.52	436.17 ±105.61

N=7, RBC**,** Red blood cell; PCV, Packed cell volume; HGB, Haemoglobin; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration; MCV, Mean corpuscular volume; WBC, White blood cell; PLT, Platelet count. *= significance at $P<0.05$ compared with normal control group using Bonferroni Post Hoc test and ANOVA

4.6 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Relative Organ Body Weight in Swiss Albino Mice

The results did not show any significant difference between the groups (Table 4.6).

Table 4.6: The effect of Quinine-Artesunate Co-administration and their Separate Therapy on Relative Organ Body Weight in Swiss albino mice

Group	Mean Values ± SEM of Relative Organ Body Weight (%)		
	Kidney	**Liver**	**Spleen**
Control	1.45 ±0.08	4.68 ±0.16	0.59 ±0.08
Quinine	1.31 ±0.08	4.69 ±0.34	0.55 ±0.10
Artesunate	1.45 ±0.09	4.86 ±0.25	0.89 ±0.12
Quinine+ Artesunate	1.27 ±0.08	4.59 ±0.25	0.53 ±0.18

N=7; SEM: standard error of mean

ANOVA and Bonferroni Post Hoc t-test

4.7 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Random Blood Glucose Level in *P. berghei* Infected Mice

The results compared across each group for differences in glucose level on the days of treatment did not show any significant difference in the Random Blood Glucose level of the groups between day 1, day 5 and day 6. A statistically insignificant increase in RBS day 1 in infected group compared to the normal control group and a significant increase when compared to Artesunate group ($P<0.05$). There was no significant difference between the groups on day 5 and day 6 (Table 4.7).

Table 4.7: The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Random Blood Glucose Level in *P. berghei* Infected Mice

Treatment Group	Mean Values ±SEM of Glucose Level (mg/dL)				
	RBS Day1	RBS Day 5	% Reduction	RBS Day 6	% Reduction
Normal Control	110.80 ±16.24	123.00 ±3.69	-11.01	111.40 ±4.47	9.43
Infected control	179.66 ±6.33*	150.50 ±16.50	16.23	142.00 ±22.00	5.65
Quinine	126.40 ±19.93	106.20 ±9.86	15.98	101.40 ±9.47	4.52
Artesunate	124.20 ±7.20	109.20 ±6.48	12.08	100.60 ±8.08	7.88
Quinine + Artesunate	138.50 ±10.53	126.25 ±7.95	8.84	126.50 ±7.03	-0.19

*= $P<0.05$ compared to Artesunate; N=5, SEM=standard error of mean, RBS, Random Blood Sugar; repeated measures ANOVA and Bonferroni

4.8 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Fasting Blood Glucose Level in *P. berghei* infected mice

Glucose levels in each group were compared across the days and between the groups. A significant decrease ($P<0.05$) in the blood glucose level of quinine group alone was found on the 7th day of treatment compared to the 4th day. There was no significant difference in the blood glucose levels across the days of other groups. On the 2nd day, a significant increase in FBS was found in infected control compared to the normal control and artesunate groups ($P<0.05$), a significant increase in FBS was also found in the quinine group when compared to the normal control and Artesunate groups. There was a significant increase in FBS on the 4th day of quinine and quinine-artesunate groups compared to the normal control group ($P<0.05$) and a significant decrease when Artesunate was compared to the normal control, infected control and quinine groups on the 7th day ($P<0.05$) (Table 4.8).

Table 4.8: The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Fasting Blood Glucose Level in *P. berghei* Infected Mice

Group	Mean Values ± SEM of Glucose Level (mg/dL)			
	FBS Day 2	FBS Day 3	FBS Day 4	FBS Day 7
Normal control	90.00 ±9.15	79.60 ±6.65	76.60 ±3.96	74.60 ±7.43^{c}
Infected control	147.00 ±15.18$^{\#}$	113.67 ±14.53	126.33 ±10.89	100.50±20.50 ^{c}
Quinine	138.60 ±9.91$^{\#}$	103.00 ±7.30	127.40 ±9.014^{a}	86.40 ±3.89* ^{c}
Artesunate	92.80 ±7.05	95.00 ±10.48	75.20 ±15.03	37.20 ±10.95
Quinine+ Artesunate	109.75 ±7.58	110.50 ±13.74	101.75 ±3.38^{a}	54.75 ±6.60

N=5, FBS**:** Fasting Blood Sugar; SEM: standard error of mean $^{\#}$= Significant at $P<0.05$ compared to Normal control and Artesunate groups; * = $P<0.05$ compared to Day 4, ^{a}= $P<0.05$ compared to Normal control, ^{c}=$P<0.05$ compared to Artesunate group using repeated measures ANOVA and Bonferroni Post Hoc test

4.9 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Liver Function Test in *Plasmodium berghei* Infected Mice

A significant increase ($P<0.01$) was found in the Alanine amino transferase (ALT) levels of quinine group compared to the normal control group. However, the study showed insignificant increases in the ALT levels when the normal control group was compared with the infected control, Artesunate and Quinine-Artesunate groups. An insignificant increase in the Alkaline phosphatase (ALP) of the Quinine, Artesunate and Quinine-Artesunate co-administration group was found when compared to the normal control group. No significant difference was observed in Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) level when compared between the groups, although there was an insignificant increase in ALP levels of quinine, artesunate and quinine-artesunate groups compared to the normal and infected control groups (Table 4.9).

Table 4.9: The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Liver Function Test in *P. berghei* Infected Mice

Group	Mean Values ± SEM of Liver Biomarkers (IU/L)		
	ALT	ALP	AST
Normal control	1372.00 ±38.41	1613.00 ±78.26	1322.60 ±26.25
Infected control	1630.00 ±18.00	1610.00 ±24.00	1431.50 ±5.50
Quinine	1652.80 ±23.96*	1850.00 ±32.34	1435.60 ±36.68
Artesunate	1539.20 ±66.77	1852.00 ±53.39	1418.20 ±13.06
Quinine+ Artesunate	1440.00 ±60.43	1769.25 ±64.57	1456.25 ±53.47

N=5, ALT, Alanine amino transferase; ALP, Alkaline phosphatase; AST, Aspartate amino transferase; SEM: standard error of mean; * = Significant at $P<0.01$ compared to normal control group using ANOVA and Bonferroni Post Hoc test

4.10 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Oxidative Stress Biomarkers in *P. berghei* Infected Mice

A significant increase in Malondialdehyde (MDA) concentration level was observed in infected control ($P<0.05$), Quinine ($Ps<0.01$), Artesunate ($P<0.01$) and Quinine-Artesunate co-administration ($P<0.05$) groups when compared to the normal control group. The study showed a significant decrease in Superoxide dismutase level of infected control ($P<0.01$), Quinine ($P<0.001$), Artesunate ($P<0.0001$) and Quinine-Artesunate co-administration ($P<0.0001$) groups when compared to the normal control group and a significant decrease was found when Quinine-Artesunate co-administration group was compared to quinine group ($P<0.05$). A significant decrease was found in the glutathione peroxidase levels of Quinine-Artesunate co-administration ($P<0.01$), Quinine ($P<0.01$) and Artesunate ($P<0.0001$) groups when compared to the normal control group. A significant decrease was also found in the catalase level of Quinine-Artesunate ($P<0.01$), quinine ($P<0.0001$), artesunate ($P<0.0001$) and infected control ($P<0.05$) groups compared to the normal control group (4.10).

Table 4.10: The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Oxidative Stress Biomarkers Test in *P. berghei* Infected Mice

Group	Mean Values ± SEM of Oxidative Stress Biomarkers (IU/L)			
	MDA	**SOD**	**GPX**	**CAT**
Normal control	1.14 ±0.08	2.46 ±0.05$^{\#\#}$	61.40 ±1.17	54.20 ±1.07
Infected control	1.65 ±0.05*	1.85 ±0.15**	55.00 ±1.00	46.00 ±2.00*
Quinine	1.62 ±0.07**	1.96 ±0.07***$^{\#}$	53.00 ±1.92**	43.60 ±1.29****
Artesunate	1.68 ±0.07**	1.70 ±0.07****	49.00 ±1.52****	44.40 ±1.23****
Quinine+ Artesunate	1.60 ±0.11*	1.63 ±0.06****	52.00 ±0.71**	45.50 ±1.32**

N=5, MDA, Malondialdehyde; SOD, Superoxide dismutase; GPX, Glutathione peroxidase; CAT, Catalase. Significant at *= $P<0.05$, **=$P<0.01$, ***=$P<0.001$ and ****=$P<0.0001$ when compared with normal control group, #= $P<0.05$ and ##= $P<0.01$ compared with Quinine-Artesunate group using ANOVA and Bonferroni Post Hoc test

4.11 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Relative Organ Body Weight of *P. berghei* Infected Mice

This study showed a significant increase in the liver-body weight ratio of infected control ($P<0.0001$), Quinine ($P<0.0001$) and Artesunate ($P<0.05$) when compared with that of the normal control group, but the increase for the co-administration group was not significant when compared with the normal control group. A significant decrease ($P<0.0001$) was seen in the normal control, quinine, artesunate and Quinine-Artesunate groups when compared with the infected control group. The study did not show any significant difference in the liver-body weight ratio of the co-administration group when compared to the normal control group. No statistical difference was seen in the relative kidney-body weight when compared between the groups, but a significant increase was seen in the relative spleen-body weight of the infected control ($P<0.01$) and quinine ($P<0.0001$) groups when compared to normal control group. A significant increase was found when quinine group was compared to normal control ($P<0.0001$), artesunate ($P<0.05$) and Quinine-Artesunate ($P<0.01$) groups (Table 4.11).

Table 4.11: The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Relative Organ Body Weight of *P. berghei* Infected Mice

Group	Mean Values ± SEM of Organ Body Weight Ratio (%)		
	Liver	**Kidney**	**Spleen**
Normal control	4.31 ±0.22$^{\#}$	1.39 ±0.15	0.71 ±0.13^{c}
Infected control	10.42 ±0.29***	1.45 ±0.05	2.15 ±0.15**
Quinine	6.81 ±0.41***$^{\#}$	1.45 ±0.06	2.29 ±0.25***
Artesunate	5.83 ±0.37*$^{\#}$	1.59 ±0.08	1.37 ±0.17^{a}
Quinine + Artesunate	5.63 ±0.23$^{\#}$	1.39 ±0.03	1.13 ±0.09^{b}

N=5, SEM= standard error of mean, Significant at *=P<0.05, **=P<0.01 and *** =P<0.0001 compared with normal control group, #=P<0.0001 compared with infected control, a=P<0.05, b=P<0.01 and c=P<0.0001 compared with quinine group using ANOVA and Bonferroni Post Hoc test

4.12 The Effect of Quinine-Artesunate Co-administration on Acetic Acid Induced Writhes in Mice

A significant decrease in the number of writhes of Artesunate + Ibuprofen group was seen when compared to the normal Ibuprofen group ($P<0.05$). Quinine + Ibuprofen, Quinine-Artesunate + Ibuprofen co-administration and Ibuprofen alone groups showed insignificant decrease in number of writhes when compared to the normal control group. The study did not show any significant difference when the groups were compared to the normal control group.

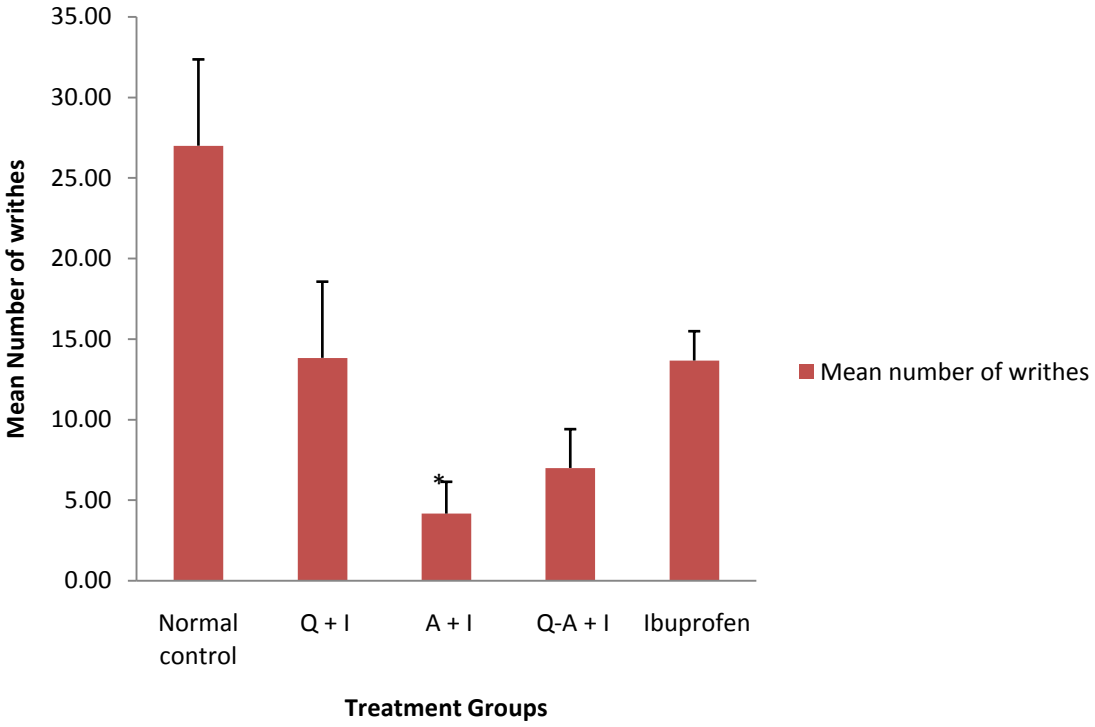


Figure 4.1: The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Acetic Acid Induced Writhes in Mice

N=7, * = significant at $P<0.05$; using ANOVA and Bonferroni Post Hoc test
Q= Quinine, A= Artesunate, I= Ibuprofen

4.13 The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Brewer's Yeast Induced Pyrexia in Rats

In normal control group, no significant difference was observed across the time. In Quinine + Ibuprofen group, a significant decrease in temperature was found at the 4th hour post treatment compared to the 0 hour ($P<0.01$) and 2nd hour ($P<0.05$). A significant increase in pyrexia was observed in the Artesunate + Ibuprofen group at the 0 hour compared to the basal temperature ($P<0.05$). Quinine-Artesunate + Ibuprofen group did not show any significant difference across the time. Ibuprofen group showed significant decrease in temperature when the 0 hour was compared to the 2nd hour ($P<0.001$) and 4th hour ($P<0.05$) and a significant decrease when 2nd hour was compared with the 3rd hour. The result showed significant decrease in pyrexia after 2nd hour in Artesunate + Ibuprofen, Quinine-Artesunate + Ibuprofen and Ibuprofen groups ($P<0.01$) when compared to the normal control group. At the 4th hour, the result showed significant decrease in pyrexia in Artesunate + Ibuprofen ($P<0.01$), Quinine + Ibuprofen ($P<0.05$) Quinine-Artesunate + Ibuprofen ($P<0.05$) and Ibuprofen ($P<0.05$) groups compared to the normal control group (Table 4.12).

Table 4.12: The Effect of Quinine-Artesunate Co-administration and their Separate Therapy on Brewer's Yeast Induced Pyrexia in Wistar Albino Rats

Treatment Groups	Mean Values ±SEM of Temperature (°C)					
	Basal Temp	0 Hour	1st Hour	2nd Hour	3rd Hour	4th Hour
Normal Control	36.63±0.36	38.17±0.47	38.06±0.25	38.52±0.23	38.12±0.29	38.34±0.24
Quinine+ Ibuprofen	36.42±0.32	38.30±0.19*	37.73±0.20	38.00±0.15$^{\#}$	37.78±0.12	37.37±0.16**$^{\#}$
Artesunate+ Ibuprofen	36.18±0.54	37.72±0.46*	37.15±0.47	37.17±0.34	37.08±0.33	37.03±0.22
Quinine-Artesunate +Ibuprofen	36.75±0.27	38.20±0.28	37.53±0.17	37.12±0.18	37.33±0.28	37.32±0.22
Ibuprofen	36.85±0.23	38.45±0.14*	37.23±0.40	37.18±0.13$^{\#\#}$	37.85±0.11^{a}	37.35±0.12*

N=6, TEMP: temperature; SEM: standard error of mean; *=P<0.05, **=P<0.01 vs. basal temperature; $^{\#}$=P<0.05, $^{\#\#}$=P<0.001 vs. 0 hour; ^{a}=P<0.05 vs. 2nd hour Repeated measures ANOVA and Bonferroni Post Hoc test

CHAPTER FIVE

DISCUSSIONS

Artemisinin-based combination therapy is the most important treatment in the chemotherapy of malaria. The mechanism of action of Artemisinin derivatives depends on the alkylation of plasmodium-specific proteins that prevents the hemozoin production and consequently causes the release of free iron that is toxic to the parasite (Souza *et al*., 2012). This study evaluated the effect of Quinine-Artesunate co-administration on blood glucose concentration as well as determined the recovery in altered biochemical variables, oxidative stress and haematological variables. This study also investigated the action of Quinine-Artesunate co-administration during concurrent administration with Ibuprofen when administered for the treatment of pain and pyrexia.

Studies have shown the efficacy of concurrent administration of intravenous quinine and Artesunate in chemotherapy of malaria (Bartoloni, 2010). The fasting blood glucose levels did not show any significant difference across the days within the groups. In the oral glucose tolerance test, Quinine group showed more significant decrease ($P<0.001$) at time 120 minute compared to time 30 minute than other groups. A slight significant decrease ($P<0.05$) in blood glucose level was found in artesunate group at time 120 minute compared to time 30 minute. Quinine-Artesunate co-administration group did not show any significant difference in blood glucose level across the time. The co-administration group did not show any effect on glucose reduction compared to the quinine and artesunate group. In *P. berghei* infected animals, no significant difference in

Random blood glucose levels across the groups was seen in all the groups, although a significant increase in the Random blood glucose level of infected control ($P<0.05$) was found when compared to the normal control group. This could be as a result of the insulin resistance in uncomplicated malaria of which the basal glucose is usually increased (Binh *et al*., 1997). In fasting blood glucose level, only quinine group showed a significant reduction ($P<0.05$) in blood glucose levels between day 4 and day 7 of drug administration. However, Quinine-Artesunate co-administration group did not show significant decrease in blood glucose level across the days of treatment when compared to the infected control group and groups treated with quinine and artesunate singly. This suggests that the co-administration reduced the risk of hypoglycaemia supposedly caused by the parasite or individual treatment groups.

The changes in the liver enzyme system have been used clinically in evaluating the toxicity of any extraneous substance to the living system. This is so, because, any derangement of biochemical processes in experimental animals due to the presence of a xenobiotic (drug) would reflect increase or decrease in the activity of such enzymes including AST, ALT and ALP used as indicators of liver injury (Edet *et al*., 2011). In this study, the ability of quinine and Artesunate co-administration on reduction of hepatotoxicity as compared to the control groups and the groups administered quinine and artesunate alone groups were investigated. In healthy animals, a significant increase in AST levels of group was found in quinine group compared to the Artesunate group. A significant increase was observed in the ALT level of quinine group ($P<0.01$) compared to the normal control group. This is in line with the documented report of Quinine

induced hepatotoxicity following ingestion of first dose (Farver and Lavin, 1999). The ALT level of the Quinine-Artesunate co-administration group was not significantly higher than the normal control group signifying its lesser damage to the liver compared to the other infected groups. The co-administration did not cause increase in ALT concentration (liver damage) that may be caused by quinine and the malaria parasite. There was no significant difference in the ALP between the groups.

In line with the report of Erel *et al*, (2001), activities of antioxidant enzymes such as the superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) in animals with malaria were reduced while lipid peroxidation of platelets (by measuring MDA) were elevated. Oxidative stress biomarkers were investigated on healthy animals, this showed no significant difference between the groups. In parasitized animals, a significant increase in lipid peroxidation (MDA) was observed in all the treated groups when compared to the normal control group. This could be as a result of the treatment and not the parasite, since the infected control did not show significant decrease compared to the normal control group. A significant decrease was found in Quinine-Artesunate co-administration group in SOD when compared with quinine ($P<0.05$) and normal control ($P<0.01$) groups. Artesunate group showed significantly higher values ($P<0.0001$) of antioxidant enzymes (GPX and CAT) compared to the normal control group. This is in line with artesunate's documented report of being associated with oxidative stress. Quinine group showed significant decrease in the antioxidant enzymes compared to the normal control group. No significant difference was seen when Quinine-Artesunate, Quinine and Artesunate groups were compared to the infected control group.

The co-administration showed a more reduced MDA values than the treated and infected control groups. This suggests that the co-administration did not induce lipid peroxidation (MDA) elevation and possesses potential effect on lipid peroxidation compared with the infected and other treated (individual drug) groups. The infected control group showed slightly significant decrease ($P<0.05$) in antioxidant enzymes (SOD and CAT) levels compared to the normal control group, it did not show significant difference on the GPX levels compared to the normal control group. The decrease in antioxidant enzymes in treated groups may not be necessarily due to the malaria parasite alone since the infected control group has a lesser reduction.

Generally in toxicological studies, relative organ body weight changes are often associated with treatment related effects (Sellers *et al*., 2007). Liver weight elevation is associated with potent hepatic enzyme-inducing compounds, while changes in kidney weight are linked to renal toxicity, tubular hypertrophy and chronic progressive nephropathy (Sellers *et al*., 2007). In healthy animals, no significant difference was seen compared between groups. In parasitized animals, a significant decrease in the relative liver-body weight was observed in quinine, artesunate and Quinine-Artesunate groups when compared with the infected control group. This suggests that the treatments reduced the risk of liver toxicity caused by the parasite. A significant increase in the relative liver-body weight was observed in infected control, quinine and artesunate groups when compared with the normal control group. Malaria is usually involved in hepatosplenomegally i.e. enlargement of the liver and spleen, this could be the reason for increment in the weight of the liver of these groups infected with malaria. Although, with

treatment especially Quinine-Artesunate co-administration, there was no significant decrease in the weight of the liver compared to the normal control group. The relative organ body weight of the kidney does not show any significant difference when compared between the groups. Although, insignificant increase was seen in infected control, quinine and Artesunate groups compared to the normal control group. Quinine-Artesunate co-administration showed the same mean value with the normal control group suggesting that the co-administration group did not affect the kidney-body weight of the animals. A significant increase was found in the relative organ-body weight of the spleen of the infected control and quinine group compared to normal control group. The relative body weight of the spleen of Quinine treated group was significantly increased compared to normal control, artesunate and Quinine-Artesunate groups. However, Quinine-Artesunate group did not show significant difference compared to normal control group.

It is important to assess the haematological parameters of experimental animals in order to help in determining any deleterious effect of foreign compounds (e.g. drugs) on the blood of the animals. In healthy animals, the effect of Quinine-Artesunate co-administration on haematological parameters was investigated. Artesunate group showed a significant increase ($P<0.05$) in the Mean Corpuscular Haemoglobin Concentration (MCHC) level when compared with the normal control group. The MCHC is a calculated mean value of the amount of haemoglobin in red blood cell. Its increase is usually seen in lipemia, obese persons or infants. No significant difference was observed in other haematological indices between the groups.

The acetic acid induced writhes method, also called the abdominal constriction response is a procedure sensitive to local peripheral receptors used to evaluate peripherally acting analgesics. It is associated with prostanoids in general, such as increase levels of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids; and lipoxygenase products (Koster *et al*., 1959; Pateh *et al*., 2011).

The data presented here showed that Artesunate concurrently administered with ibuprofen showed a significant decrease ($P<0.05$) in the number of writhes when compared with Ibuprofen group. This is in agreement with the report of Mannikoth *et al* (2012) that documented the analgesic effect of Artesunate. Quinine-Artesunate co-administration concurrently administered with Ibuprofen showed an insignificant decrease in the number of writhes in the animals compared to Ibuprofen and Normal control groups. There was also an insignificant decrease in the number of writhes of quinine + Ibuprofen and Ibuprofen groups compared to the normal control group.

Fever is an elevation of core body temperature above normal. A normal adult oral temperature is 37°C, above 38°C is considered significant. Febrile response (fever) is a complex physiologic reaction to disease involving a cytokine-mediated rise in body temperature, generation of acute-phase reactants and activation of numerous physiologic, endocrinologic and immunologic systems (Dalal and Zhukovsky, 2006). A significant decrease in pyrexia was found at the 2nd hour ($P<0.05$) and 4th hour ($P<0.001$) post treatment compared to the 0 hour in the quinine + Ibuprofen group. A significant reduction in pyrexia was seen in Ibuprofen group at the 2nd hour ($P<0.001$) compared to

the 0 hour. Quinine-Artesunate co-administration did not show significant reduction in pyrexia across the time.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

This study was carried out to determine the effect of Quinine-Artesunate co-administration on some biochemical parameters on both healthy and *Plasmodium berghei* infected albino mice. It was also to investigate the analgesic and anti pyretic effect of Ibuprofen when concurrently administered with Quinine and Artesunate. Both quinine and artesunate were co-administered in *Plasmodium berghei* infected and healthy albino mice for seven days and on the eighth day the animals were euthanized and blood samples, liver, spleen and kidney were collected for investigation. The results however, showed that the co-administration in many cases reduced the level of toxicity shown by either drug (quinine/artesunate) when administered singly. Hypoglyacemia and liver toxicity caused by quinine alone and the oxidative stress caused by artesunate were absent when both drugs were co-administered. The concurrent administration of quinine and artesunate with Ibuprofen produced little effect on pyrexia and algesia.

6.2 Conclusion

Quinine and Artesunate are both effective drugs used in the treatment of malaria. However, the percentage reduction in blood glucose concentration of the co-administration was lower than that of the Quinine group.

The co-administration showed a lower liver enzyme level concentration on the most important liver enzyme biomarker used in checking liver damage (Alanine transaminase, ALT). Thus, this suggests that the co-administration had a better or less adverse effect on the liver. The co-administration had a lower Malondialdehyde level compared to the infected control and individual drugs groups. This suggests that the co-administration has a better effect on lipid peroxidation than when the drugs are administered separately.

The co-administration does not show any significant difference in both analgesic and anti-pyretic activity when compared to the standard group administered drug, Ibuprofen. It can be concluded therefore that the co-administration concurrently treated with Ibuprofen does not affect the action of Ibuprofen.

Conclusively, quinine and artesunate are better co-administered than administering the individual drugs alone and can be concurrently administered with Ibuprofen.

6.3 Recommendations

1. Quinine or other anti-malarials should be co-administered with other Artemisinin derivatives to investigate the efficacy and adverse effects.
2. The effect of Quinine-Artesunate co-administration on renal indices (electrolytes, urea and creatinine) and histopathology of the organs should be investigated.
3. The concurrent administration of the co-administration and other analgesic agents and anti-diabetic drugs in case of patients with diabetes should also be

investigated since malaria also occurs in diabetic patients to investigate the interaction.

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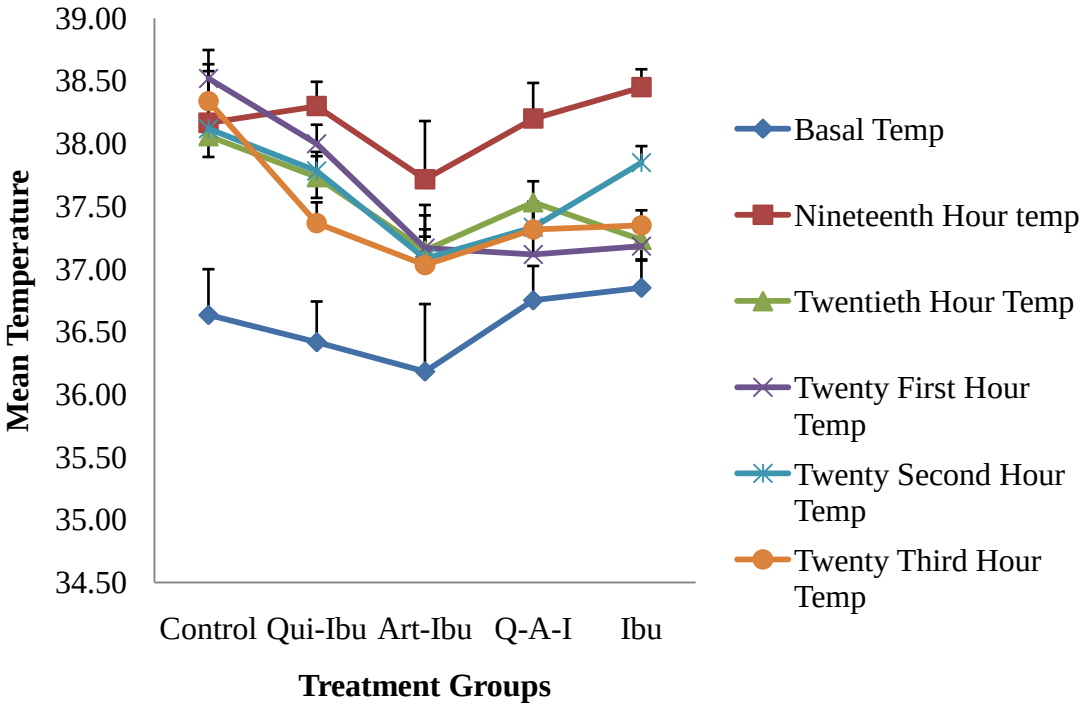
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APPENDIX

Appendix 1: Figure 4.1: The effect of Quinine-Artesunate Co-administration on Acetic Acid Induced Writhes in mice

GROUP	Mean Number of Abdominal Writhes (±SEM)	% INHIBITION
Normal Control	27.00 ±5.36	-
Quinine + Ibuprofen	13.83 ±4.73	49
Artesunate + Ibuprofen	4.17 ±1.97*	85
Quinine-Artesunate+Ibuprofen	7.00 ±5.38	74
Ibuprofen	13.67 ±1.82	49

Appendix 2: The effect of Quinine-Artesunate co-administration on Brewer's yeast induced pyrexia in albino rats



Appendix 3: Contents of pelletized growers feed

The pelletized growers feed used in feeding the animals contains the following ingredients

Cereals/Grains, Vegetable Protein, Premix (Vitamins/Minerals), Essential amino acids, Salt, Antioxidant, Anti-toxins, Prebiotic, Enzymes

Crude protein	15%
Fat	7%
Crude fiber	10%
Calcium	1.0%
Available phosphorus	0.35%
Metabolisable energy	2550 Kcal/Kg