EFFECTS OF ANTIMALARIAL DRUGS ON OESTROUS CYCLE AND UTERINE CONTRACTILITY IN RATS

BY

Maryam wawata USMAN

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

FACULTY OF PHARMACEUTICAL SCIENCES

AHMADU BELLO UNIVERSITY

ZARIA, NIGERIA

NOVEMBER, 2014

TITLE PAGE

EFFECTS OF ANTIMALARIAL DRUGS ON OESTROUS CYCLE AND UTERINE CONTRACTILITY IN RATS

Maryam Wawata USMAN

B. Pharm (ABU) 2000

M.SC/PHARM.SCI/05365/2009-2010

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES

AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF A MASTER DEGREE IN PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,

FACULTY OF PHARMACEUTICAL SCIENCES,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

NOVEMBER, 2014

DECLARATION

I declare that this Thesis entitled "effect of antimalarial drugs on oestrous cycle and uterine contractility in 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 was previously presented for another degree or diploma at this or any other institution.

USMAN Maryam Wawata _____ _____

Signature Date

CERTIFICATION

This thesis entitled "**EFFECTS OF ANTIMALARIAL DRUGS ON OESTROUS CYCLE AND UTERINE CONTRACTILITY IN RATS"** by MARYAM WAWATA USMAN meets the regulations governing the award of the degree of Master of Science in Pharmacology of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

Prof. H.O. Kwanashie — Signature _____
Chairperson, Supervisory committee

Date _____

Dr. J.I. Ejiofor — Signature _____
Member, Supervisory committee

Date _____

Dr. A. U. Zezi — Signature _____

Head, Department of Pharmacology — Date _____

Prof. A. Z. Hassan — Signature _____
Dean, School of Postgraduate Studies

Date _____

DEDICATION

In loving memory of my father late Retired Captain Usman Wawata and my sister late Adama Usman Wawata for your love, support and words of encouragement all the way.

ACKNOWLEDGEMENTS

I am highly indebted to my team of supervisors Prof. H. O Kwanashie and Dr J. I. Ejiofor for their time, concern and valuable contributions towards the success of my work.

I deeply appreciate the assistance given to me by the entire staff of the Department of Pharmacology and Therapeutics, most especially, Prof. J.A. Anuka, Dr T.O. Olurishe, Dr, M. G. Magaji, and Dr M. Yerima for all their advice and guidance from which I benefited tremendously during the course of my study.

Special thanks goes to Mr I. Adamu, Mr J. Kono, Mr J. Ose, Mr S. Nasiru, Mr M. Muazu, Mr H. Idris, Mr H. Aliyu, Mr U. Mohammed, Mr D. Tukur, Mr U.S Shehu, Mr B. Abubakar, Mr Y. Dari and the entire staff of Pharmacology and Therapeutics, for their kindness, assistance, encouragement and useful contributions.

My gratitude goes to Mallam Adamu of the Department of Pharmacognosy and Drug Development and Mallam Mohammed Ibrahim and Mallam M. Sani of Microbiology unit of the University Health Services A.B.U, Zaria for their time and support in making my work a success.

Not forgetting my husband Prof. Magaji Garba, my children Aisha, Maryam, Auwab and Abubakar for being patient, understanding and above all praying for me all the way; thank you all. My deepest gratitude goes to the Wawata's and Sami's family especially my kid sister Zuwaira Usman Wawata; without all of you I wouldn't have been where I am today. THANKS so much for all the love, care and understanding. You are the best.

Not forgetting my great friends in and outside M.Sc. class who made it a walk to remember, thank you for the priceless assistance and encouragement. You are the best.

ABOVE ALL, I THANK THE ALMIGHTY ALLAH FOR HIS INFINITE MERCY AND FOR GIVING ME THE HEALTH AND STRENGTH TO UNDERTAKE AND COMPLETE THIS WORK. TO ALLAH ARE THE GLORY, HONOUR, POWER AND MAJESTY.

ABSTRACT

Malaria is increasingly a health burden globally with children under the age of 5 and pregnant women being the most vulnerable. Several antimalarials are available in the market today however; the safety data in pregnancy is inadequate especially the first trimester of pregnancy. The study was carried out in both pregnant and non-pregnant wistar rats to evaluate the effect of these antimalarial drugs on the oestrous cycle as well as on the uterine smooth muscle contractility.

All the antimalarial drugs in this study (AT 3.2 mg/kg loading dose and 1.6 mg/kg maintenance dose for 7 days; CQ 2.5 mg/kg every 4 hours; QN 20 mg/kg loading dose and 10 mg/kg maintenance dose 8 hourly for 7 days and SP 25 mg/1.25 mg/kg once); showed a desynchronization of the normal oestrous cycle. Proestrus phase was increased by all the antimalarial drugs except for AT, oestrus phase was decreased by all but significantly by QN and metoestrus was increased by all except SP which decreased it significantly while the dioestrus phase, a decrease was observed. On isolated uterine smooth muscle of pregnant rat, artemether (AT, 16 µg/ml – 1280 µg/ml) Chloroquine (CQ, 4 mg/ml – 32 mg/ml), quinine (QN 100 ng/ml – 800 ng/ml) and sulphadoxine /pyrimethamine (SP, 100 µg/ml – 400 µg/ml), had no agonist effect on the uterus but AT (16 µg/ml – 1280 µg/ml), CQ (16 mg/ml) and SP (100 µg/ml) produced a reduction in oxytocin induced contraction of the pregnant uterus. In conclusion, this study showed that these antimalarial drugs caused a desynchronization of the normal oestrous cycle suggesting a possible effect on the menstrual cycle in women of child bearing age while the reduction in the oxytocin induced contraction could translate to ill effect especially in late pregnancy and during labour.

TABLE OF CONTENTS

Title Page - - - - - - - - i

Declaration - - - - - - - - ii

Certification - - - - - - - - iii

Dedication - - - - - - - - iv

Acknowledgements - - - - - - - - v

Abstract - - - - - - - - vii

Table of contents - - - - - - - - viii

List of figures - - - - - - - - xii

CHAPTER ONE

1.0 INTRODUCTION - - - - - - 1

1.1 Background to the Study - - - - - 1

1.1.1 Malaria - - - - - - - - 1

1.1.2 Antimalarial Drugs - - - - - - 3

1.1.3 Oestrous Cycle in Rats - - - - - 4

1.1.4 Uterine Smooth Muscle and Contractility - - - 5

1.2 Statement of the Research Problem - - - - 6

1.3 Justification for the Study - - - - - 7

1.4 Theoretical Framework - - - - - 8

1.5 Aim and Objectives - - - - - - 8

1.5.1 Aim - - - - - - - - 8

1.5.2 Objectives - - - - - - - 9

1.6 Hypothesis - - - - - - - 9

CHAPTER TWO

2.0 LITERATURE REVIEW - - - - - 10

2.1 Malaria - - - - - - - 10

2.1.1 Incidence and prevalence of malaria - - - - 10

2.1.2 Causative agent and vector of malaria - - - 11

2.2 Drug Treatment of Malaria - - - - - 16

2.2.1 Importance of malaria chemotherapy - - - - 16

2.2.2 Antimalarial drugs - - - - - - 16

2.3 Malaria Chemotherapy in Pregnancy - - - - 20

2.3.1 Malaria in pregnancy - - - - - - 20

2.3.2 Malaria in early childhood - - - - - 22

2.3.3 Clinical status of antimalarials in pregnancy - - - 22

2.4 Pharmacology of Some Antimalarial Drugs - - - 24

2.4.1 Quinine - - - - - - - 24

2.4.2 Chloroquine - - - - - - - 26

2.4.3 Sulphadoxine/Pyrimethamine - - - - - 27

2.4.4 Artemether - - - - - - - 29

2.5 The Oestrous Cycle - - - - - - 32

2.5.1 Rat oestrous cycle - - - - - - 32

2.5.2 Factors affecting the oestrous cycle - - - - 33

2.5.3 Different stages of oestrous cycle and cell types - - 34

2.5.4 Various methods of determining the oestrous cycle in rats - 39

2.5.5 Oestrous cycle of human - - - - - 40

2.6 The Uterus - - - - - - - 41

2.6.1 Anatomical and physiological consideration of the uterus - 41

2.6.2 Drugs and the uterus - - - - - - 42

CHAPTER THREE

3.0 MATERIALS AND METHODS - - - - 48

3.1 Location of study - - - - - 48

3.1.1 Experimental animals- - - - - - 48

3.1.2 Materials - - - - - - - 48

3.1.3 Drugs and Diluents - - - - - - 49

3.2 Methods - - - - - - - 50

3.2.1. Oestrous cycle determination and effect of antimalarial drugs 50

3.2.2 Isolated uterine tissue and effect of antimalarial drugs - 51

3.3 Statistical Analysis - - - - - - 52

CHAPTER FOUR

4.0 RESULTS - - - - - - - 52

4.1 Effects of the Antimalarial Drugs on Oestrous Cycle - 53

4.1.1 Artemether - - - - - - 53

4.1.2 Chloroquine - - - - - - 55

4.1.3 Quinine - - - - - - 57

4.1.4 Sulphadoxine/ pyrimethamine - - - - - 59

4.1.4 Comparison of the effects of the antimalarial on the stages of the oestrous cycle - - - - - 62

4.2 Effects of the Antimalarial Drugs on Contractility of the Pregnant Rat uterus - - - - - - 65

4.2.1 Oxytocin- - - - - - - - - 65

4.2.2 Artemether- - - - - - - - - 66

4.2.3 Chloroquine- - - - - - - - - 68

4.2.4 Quinine- - - - - - - - 70

4.2.5 Sulphadoxine/ pyrimethamine- - - - - - 71

CHAPTER FIVE

5.0 DISCUSSION - - - - - - 72

5.1 Effect of Antimalarials on the Oestrous Cycle in Wistar Rats 72

5.2 Effect of Antimalarials on Uterine Contractility of Wistar Rats 74

CHAPTER SIX

6.0 Conclusion and Recommendation - - - - 76

6.1 Conclusion - - - - - - - 76

6.2 Recommendation- - - - - - 76

References - - - - - - - 78

Appendix I - - - - - - - 93

Appendix II - - - - - - - 94

Appendix III - - - - - - - 95

Appendix IV - - - - - - - 96

Appendix V - - - - - - - 97

Appendix VI - - - - - - - 98

Appendix VII - - - - - - - 99

Appendix VIII - - - - - - - 100

Appendix IX - - - - - - - 101

Appendix X - - - - - - - 101

Appendix XI - - - - - - - 102

Appendix XII - - - - - - - 102

Appendix XIII - - - - - - - 103

Appendix XIV- - - - - - - 103

List of abbreviations - - - - - - 104

LIST OF FIGURES

Figure 2.1 The life cycle of *plasmodium* parasite in mosquito and man . - - - - - - - - 14

Figure 2.2: Photomicroscopic image of the different cell types seen in vaginal smear of rat - - - - - - - 38

Figure 4.1: Plots for daily smear obtained for Artemether over 39 days showing various stages of the oestrous cycle - - - 54

Figure 4.2: Occurrence of oestrous cycle stages in rats before, during and after Artemether administration - - - - - - 55

Figure 4.3: Plot for daily smear obtained for Chloroquine over 34 days showing various stages of the oestrous cycle - - - 56

Figure 4.4: Occurrence of oestrous cycle stages in rats before, during and after chloroquine administration - - - - - 57

Figure 4.5: Plot for daily smear obtained for Quinine over 40 days showing various stages of the oestrous cycle - - - - 58

Figure 4.6: Occurrence of oestrous cycle stages in rats before, during and after quinine administration - - - - - - 59

Figure 4.7: Plot for daily smear obtained for Sulphadoxine-Pyrimethamine over 33 days showing various stages of the oestrous cycle - - - - - - - - 60

Figure 4.8: Occurrence of oestrous cycle stages in rats before, during and after Sulphadoxine/Pyrimethamine administration - - 61

Figure 4.9: Proestrus stage after administration of the antimalarials - - 63

Figure 4.10: Oestrus stage after administration of the antimalarials - - 63

Figure 4.11 Metoestrus stage after administration of the antimalarials - 64

Figure 4.12 Dioestrus stage after administration of the antimalarials - 64

Figure 4.13: Contractile effect of Oxytocin on isolated pregnant rat uterus - 65

Figure 4.14: Effect of Artemether on the isolated pregnant rat uterus - 66

Figure 4.15: Effect of Artemether and Oxytocin on the isolated pregnant rat uterus- - - - - - - - - 67

Figure 4.16: Effect of Chloroquine on the isolated pregnant rat uterus - 68

Figure 4.17: Effect of Chloroquine effect on the isolated pregnant rat uterus 69

Figure 4.18: Effect of Quinine effect on the isolated pregnant rat uterus - 70

Figure 4.19: Effect of Sulphadoxine-Pyrimethamine effect on the isolated pregnant rat but diminishing contractile effect of oxytocin - 71

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Malaria is an infection caused by the parasite of the genus plasmodium; four distinct species of plasmodium have been identified, namely: *P. falciparum, P. vivax, P. ovale and P. malariae. P. falciparum* causes the most serious form of the disease and is responsible for over 95% of infections in sub-Saharan Africa. *P. knowlesi,* is a fifth specie recently documented to cause human infections and is presently restricted to countries of Southeast Asia (Lee *et al.,* 2011). The parasites are transmitted through the bite of an infected Anopheles mosquito and the infection is said to be endemic in tropical countries in the World. According to Chukwuocha *et al.,* (2009) malaria is among the common causes of childhood mortality in Nigeria. It is estimated that 50% of the population have at least one episode of malaria each year, while children under five have on the average, 2-4 attacks in a year. It has been estimated that each year more than 25 million women become pregnant in malaria endemic areas mostly in sub-Saharan Africa, and 75,000 to 200,000 infant deaths are attributed to malaria infection in pregnancy (Steketee *et al.,* 2001*;* WHO, 2012*).* Malaria in pregnancy causes maternal anaemia, miscarriages, and low birth weight, and in endemic countries, it is the leading cause of maternal mortality and one of the primary causes of neonatal deaths (WHO and UNICEF, 2003).

Almost all the deaths are among children younger than five years old. Other high risk groups include women during pregnancy, non-immune travellers, refugees and other displaced persons, as well as people of all ages living in areas of unstable malaria transmission (WHO and UNICEF, 2003). In highly endemic areas like Nigeria, malaria poses a serious danger to pregnant women and their unborn children. The

severity of clinical manifestations is determined by the level of immunity before pregnancy, which depends on the intensity and stability of malaria transmission. In low and /or unstable transmission areas, the degree of acquired immunity of women prior to pregnancy is low, and both mother and her foetus are at risk for the most severe consequences of the infection.

In the past, pregnant mother with her unborn child were regarded as an integral whole and the placenta as a protective barrier to the foetus (Hugh and Barber, 1981). So, drugs were administered with only the mother's immediate need in mind, not considering facts that the developing foetus is inadvertently exposed and vulnerable to agents solely meant for the mother. However, with the introduction of sensitive analytical methods for the determination of most drugs in small volumes of plasma, it was established that essentially all drugs taken by a pregnant woman can be transferred across the placenta from the mother to foetus and vice-versa (Levy, 1981). Fear of possible adverse effects rather than knowledge of definite adverse effects of drugs have become the hinges for the exclusion of pregnant women from the use of drugs (Wilson, 1975; Philip-Howard and Wood, 1996). This fear largely resulted from the thalidomide phocomelia effect of the early 1960's which also led to the withdrawal of pregnant mothers from research designs and studies. The current consensus of opinion is that no drug should be prescribed for pregnant women unless drug therapy is essential and this is in line with what has been known since 1940s (Kohen, 2004).

Pregnant mothers are also affected by the common epidemics and infections of man and sometimes they are at higher risk of the disease for which the restricted drugs are required as in malaria, where pregnant women and their infants are most vulnerable in

terms of the prevalence and intensity of the infection (Bricaire *et al*., 1991; Hoffman *et al*., 1992; Mvondo *et al*., 1992; Luzzi and Peto, 1993; Singh *et al*., 1999; Shulman, 1999; Guyatt and Snow, 2004). It might be possible that some of these drugs that are excluded for use by pregnant patients could be actually safe and beneficial. Therefore, while there is need for caution and restraint in the use of drugs during pregnancy, their specific effects need to be studied.

1.1.1 Antimalarial drugs

Antimalarial drugs are therapeutic agents for the prophylaxis and treatment of malaria. The rationale for the development and use of each agent is considered in terms of the biology of the malaria infecting parasites (Tracy and Webster Jr. 2001). Antimalarial drugs are classified based on the stages of the parasite they affect and the corresponding clinical objectives into:-

I. *Tissue schizontocides used for causal prophylaxis* e.g. chloroguanide (proguanil),

II. *Tissue schizontocides used to prevent relapse* e.g. primaquine.

III. *Schizontocides used for clinical and suppressive cure*, this group is further divided into:

- Rapid acting schizonticide e.g. chloroquine, quinine, artemisinin based compounds.
- Slow acting schizonticides e.g. pyrimethamine/sulphadoxine and doxycycline. Drugs in this sub-group are commonly used in

combination with their rapid acting counterpart (Tracy and Webster Jr, 2001).

IV. *Gametocides and Sporontocides* are also classes of antimalarial drugs which are effective against the sexual erythrocytic forms of plasmodia preventing or inhibiting formation of malaria oocytes and sporozoites thereby preventing transmission of malaria to parasite e.g. primaquine. However, antimalarial drugs are not used clinically for this purpose (Tracy and Webster Jr, 2001).

1.1.2 Oestrous Cycle in Rats

The reproductive cycle of female rats is called the oestrous cycle and is characterized by cyclic changes in the uterus, ovaries, vaginal mucosa, behaviour and hormone levels (Maeda *et al*., 2000). This short cycle of 4-5 days make the rat an ideal animal for investigation of changes occurring during the reproductive cycle (Spornitz *et al.*, 1994). There are four (4) stages which are proestrus, oestrus, metoestrus and dioestrus (Long and Evans 1922; Freeman, 1988). Ovulation (heat) occurs from the beginning of proestrus to the end of oestrous (Young *et al.*, 1941). Oestrous cycle stage can be useful in research areas besides reproduction and infectious diseases such as brain injury outcome in rodent models of cerebral ischemia (Carswell *et al.*, 1999, Carswell *et al.*, 2000). Vaginal cytology can be used for categorising female animals and the various rat's oestrous stages can be reliably identified from vaginal smears (Montes and Luque, 1988). The association between hormone levels and oestrous stages as determined by vaginal cytology is well known

(Butcher *et al.*, 1974, Maeda *et al.*, 2000) and serve as the standard for comparison and verification.

1.1.3 Uterine Smooth Muscle and Contractility

The uterine smooth muscle is characterized by a high degree of spontaneous electrical and contractile activity. Waves of decreased membrane potential with superimposed spike activity are associated with contraction. Cell-to-cell spread of excitation occurs, but electrical conduction is slow and decremental in nature. Low-resistance contacts between cells (gap junctions) greatly facilitate the spread of excitation. The number of such junctions is regulated by steroid hormones and increases in the later stages of pregnancy. Increased frequency and duration of spike activity in "pacemaker" areas and more extensive spread of excitation are associated with increases in force of contraction. In most species (including the human female), the influx of Na^{+} appears to play the primary role in depolarization. The amount of Ca^{2+} that crosses the plasma membrane during excitation is insufficient to cause contraction directly. It is sufficient, however, to trigger the release to much larger amounts of Ca^{2+} from the sarcoplasmic reticulum (Huszar and Roberts, 1982; van Breemen and Saida, 1989). Hence, the availability of extracellular Ca^{2+} (or the presence of blockers of ca^{2+}channels) strongly influences the response of uterine smooth muscle to various physiological and pharmacological stimuli. Uterine smooth muscle is unusually susceptible to endocrine influence, especially that of the oestrogens. Thus, spontaneous activity, as well as responsiveness to neurogenic, hormonal and pharmacological stimulation, increase greatly at puberty and vary thereafter, with the ovulatory cycle (Tracy and Webster Jr, 2001).

Examples of drugs that have effects on uterine activity are:

- oxytocin which stimulate both frequency and force of contractile activity of uterine smooth muscles
- Prostaglandins which are found in the ovaries, myometrium and menstrual fluid and stimulate uterine smooth muscle activity and
- The Ergometrine, which markedly increase the motor activity of the uterus increasing the force and frequency of contraction of the uterine smooth muscle. These categories of drugs are referred to as tocogenics. The other class of drugs that have effect on the uterine activity are referred to as tocolytics e.g. of drugs in this class include β_2-adrenergic receptor agonists e.g. Salbutamol, Ca^{2+} channel blockers e.g. nifedipine and magnesium sulphate (Tracy and Webster Jr, 2001).

1.2 Statement of the Research Problem

Malaria being a disease of global concern and a major cause of morbidity and mortality in sub Saharan Africa, affects children and women especially pregnant women and their foetuses, with the latter group constituting the largest population at risk of the disease (WHO and UNICEF, 2003). During pregnancy the effect of malaria can be severe anaemia, low birth weight, placental malaria and pre mature labour. The antimalarial drugs used in the treatment of malaria as well as other medications have effects that limit their use in pregnancy and lactation. Antimalarial drugs are not only used by pregnant women alone, women of child bearing age also take these classes of drugs at some point in their lives. Therefore, these groups should not be ignored when assessing the effects of these drugs on reproductive health or

status (importantly the menstrual cycle or oestrous cycle) as well as the outcome of pregnancies.

1.3 Justification for the Study

There are a lot of antimalarials in use both for prophylaxis and cure of malaria infection. Some of these antimalarials have documented effects on pregnancy and pregnancy outcome e.g. dihydroartemisinin-piperaquine combination. However, the new antimalarial used for the treatment of malaria e.g. Artemether, has been recommended to be used with caution in the first trimester of pregnancy since some foetal absorption have been observed. This goes to show that there are none or insufficient data to justify their use in pregnancy and lactation. It was reported by Nosten *et al.* (2006), that the number of pregnant women treated with antimalarials who have been included in drug pharmacokinetic studies worldwide are less than 100 and this is in agreement with the findings of Ward *et al.* (2007), who further reported the difficulty in the assessment of some adverse reactions in pregnant women that can only be identified after delivery. Furthermore, dihydroartemisinin was reported to cause reproductive impairment in male rats and an observed relative weight gain of the testis indicates that the drug may have toxic effect on the testis (Nwanjo *et al*., 2007). It is also important to determine the effects of antimalarial drugs in women of child bearing age as it will go a long way in establishing the effectiveness and safety of these antimalarials in the treatment and / or prophylaxis of malaria during menstruation as well as in pregnancy.

1.4 Theoretical Framework

The effect of the antimalarial drugs, chloroquine, artemether, quinine, and sulphadoxine/pyrimethamine combination will be studied on the oestrous cycle and the uterine activity in rats. Isolated uterine tissue of pregnant rat will be used for effect on uterine activity. Adult non-pregnant female rats will be exposed to these antimalarial drugs and observed for any effect on the oestrous cycle using the pipette vaginal lavage smearing technique. It was reported by Ejiofor (2006), that artemether may cause resorption and/or abortion of embryo when given at mid-term. If this study suggests embryo resorption then, the possible effect of this agent on the oestrous cycle should not be overlooked (a vital stage in the reproductive life of the animal). More so, Nwanjo *et al*., (2007) reported the possibility of dihydroartemisinin (DHA) causing reproductive impairment in male rats. The same study observed a relative weight gain of the testis when DHA was administered, suggesting toxic effect of DHA on the testis. Therefore, it is important to assess the effects of these antimalarial drugs on the reproductive status or health of pregnant and non-pregnant rats.

1.5 Aim and Objectives

1.5.1 Aim

To evaluate the effect of commonly used antimalarial drugs on the reproductive status and uterine contractility of wistar rats.

1.5.2 Objectives

The specific objectives were

- To determine, the effect of antimalarial on the oestrous cycle of female wistar rats.
- To investigate the effect of antimalarial on the contractility of the uterine smooth muscle of wistar rats.

1.6 Hypothesis

The antimalarial drugs, chloroquine, artemether, quinine and sulphadoxine/pyrimetha mine combinations have no effect on the oestrous cycle and uterine contractility of rats.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Malaria

2.1.1 Incidence and prevalence of malaria

Malarial is a life threatening disease caused by the parasites that are transmitted to people through the bites of infected mosquitoes (WHO, 2013). Malaria still claims a heavy toll of death and disabilities even at the beginning of the third millennium (Francesco *et al.,* 2012), ravaging much of Africa despite attempts to find lasting solution to the dreadful and deadly parasitic disease. It is largely endemic in the tropical countries e.g. Latin America and Africa where more than 40% of the world's population is residing (Danis and Gentilini, 1998). Globally, 300-500 million people are believed to contract the disease annually resulting to about 1.2-2.7 million deaths (Muentener et al., 1999; Sachs and Malaney 2002). An estimated 90% of malaria infections occur in sub-Saharan Africa with Nigeria accounting for a quarter of all malaria cases in the world (WHO, 2010). Approximately 50,000 Nigerian women die each yearly from preventable pregnancy-related complications (Erin *et al.,* 2012). Malaria infection during pregnancy poses substantial risks to the mother and her unborn child with consequences of severe anaemia, placental parasitaemia and intrauterine growth retardation which contribute to low birth weight, a principal cause of infant mortality in the African region. Prevalence of malaria in pregnancy compared to the general population is higher with 40% of all women estimated to be exposed to malaria infection during the course of pregnancy (Desai *et al.,* 2007), with immunosuppression and loss of acquired immunity to malaria being a possible reason. Yearly deaths associated with malaria in pregnancy was estimated at 75,000-200,000 (Steketee *et al.,* 2001), with severe malaria anaemia accounting for more than half of

these deaths. *Falciparum* malaria during pregnancy has long been recognised as an important determinant of low birth weight this is more marked in primigravidae, but it can extend to second and third gravidae in areas of low malaria transmission (Nosten *et al.,* 1999*).* Africans and people living in malaria stable zones develop partial immune defence after the body system is routinely exposed to malaria infection (Kurtis *et al*., 2001). This immunity as the name implies is partial and is soon lost when the individual's exposure to the sporozoite inoculation stops as a result of prolonged stay in a non-endemic region or zone. Socio-economic factors of underdevelopment in the tropics is also a factor that influences the severity, prevalence and management of malaria, particularly poverty, ignorance and inadequacy of both health structures and health care delivery as well as unavailability of effective drugs at the locations where they are needed (Chukwuani, 1999; Salako, 1999). Worthy of mention is the problem of drug resistance by the parasites to both insecticides and commonly used antimalarial drugs particularly the first line drugs.

2.1.2 Causative agent and vector of malaria

P. falciparum the predominant and notorious plasmodia specie is responsible for over 90% of malaria infection in man (Molineaux and Gramiccia, 1980). It has been reported to be of vital importance in disease transmission in Nigeria. It is virulent and deadly causing severe or complicated malaria and possessing an inherent capacity to develop resistance to antimalarial drugs (Shuler, 1985; Cowman, 1995). *P. malariae* is responsible for only 15% and *P. ovale* for less than 5% of human malaria, but both usually occur as mixed infections with *P. falciparum* (Bruce-Chwatt, 1951; Molineaux and Gramiccia, 1980). *P. vivax* is more common than *P. falciparum* as a cause of malaria in many parts of the tropics outside Africa because of genetic deficit

of the Duffy antigen receptors required by this specie for its invasion into the red blood cells (RBCs) and it only presents with benign tertian malaria (Nosten *et al*., 1999; Nicholas *et al*., 2000). Plasmodia infection is transmitted into the blood by the bite of malaria carrier vectors, the female anopheles mosquitoes which are more frequent in tropical and sub-tropical than in temperate regions. The vectors are most active at night during which they move indoors in search of blood meal for pregnancy (Gillies and De Meillon, 1968). The factors that favour rapid multiplication of the vector include: water and humidity (>60%), generous rainfall, ambient temperature of 20-30^{o}C and topography of <2,000m elevation above sea level (WHO, 1987). The female anopheles mosquito has an average life span of 4 to 6 weeks under favourable climatic conditions (WHO, 2013).

2.1.2.1 *Life cycle of the parasite and mode of transmission*

The life cycle of the plasmodia parasite is bi-phasic partly occurring in the mosquito (sexual phase) and partly in the liver and erythrocyte of the vertebrate host (asexual phase) (Bouree *et al.,* 1994). The infective mosquito harbours the sporozoites in its saliva and deposits it on the inner skin during blood meal. The sporozoites penetrate into the blood stream and undergo a series of asexual proliferation into various polymorphic forms. Other modes of malaria transmission include blood transfusions and sharing syringes. Mother to child transmission during pregnancy has also been documented, but all the modes of transmission other than via the mosquito are believed to be very rare and unimportant (Slinger *et al.*, 2001, Valecha *et al*., 2007, Chauhan *et al*., 2009). Gametocytes are the products of some intra-erythrocytic trophozoites, which are not capable of further differentiation in the blood and so tend to differentiate into male and female gametocytes. This differentiation is the sexual

phase of the plasmodia lifecycle. These gametocytes are not pathogenic and they cannot develop or transform into the pathogenic sporozoites until they are successfully deposited into the gastrointestinal tract (GIT) of mosquitoes. Therefore, they remain in the blood stream of the vertebrate host as infective reservoir or carriers until sucked out by and into the GIT of the mosquito (Bouree *et al.,* 1994; Tracy and Webster Jr, 2001). It takes about 10 days for malaria parasites in a mosquito to become infective to humans. The asexual transformations that take place in the vertebrate host consist of the various polymorphic forms of the sporogony and schizogony stages of the lifecycle. Each of the three schizontal stages (pre-erythrocytic, erythrocytic and intra-erythrocytic) of the asexual phase of the parasites has a two-step cycle that leads to the rupture and release of the next higher schizont form in a continual cyclic manner. Bursting of the trophozoites or schizonts which may occur synchronously or separately every 48 hours (tertian malaria) or 72 hours (quartan malaria) depends on the plasmodia specie. Relapse due to *P. vivax and P. ovale* are often as a result of reactivated intra-hepatic forms which tends to remain dormant or quiescent (hypnozoites) for several months or years. However, the cause(s) of relapse in *P. malariae* is not known (Lechner *et al.,* 1997; Nicholas *et al.,* 2000). The sexual forms of plasmodium parasites are particularly persistently resistant to drugs (tracey and Webster Jr., 2001). Figure 2 illustrates the lifecycle of the plasmodia parasite.

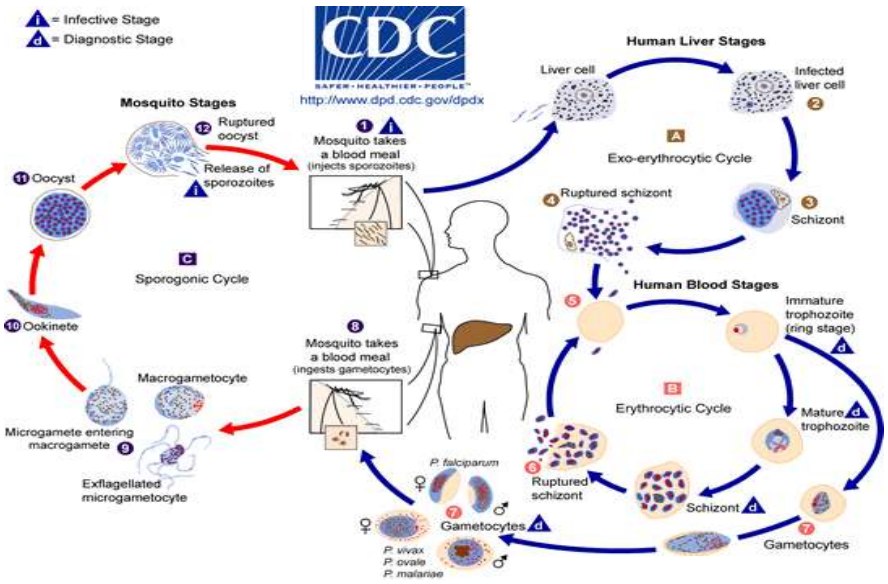


Figure 2.1 The life cycle of *plasmodium* parasite in mosquito and man (CDC, 2006).

2.1.2.2 Clinical presentation of malaria

Malaria was previously referred to as marsh fever owing to the feverish conditions and chills associated with it (Bouree *et al*., 1994). The initial invasive attack consists of typical episodes of sudden and temporary conditions of chill, fever, profuse sweating and tiredness (Bouree, 1997). The initial symptoms may subside in the absence of therapy, but may reoccur after few days, weeks, months or years (recrudescence or relapse). The molecular mechanism underlying the clinical symptoms and the host-parasite interaction of plasmodia parasites are still not clear (Kilunga-kubata *et al*., 1998). It has been found that the clinical symptoms of malaria are triggered by the release of toxic pyrogenic substances from both the plasmodia and the host cells (Bouree *et al*., 1994). The parasite releases several antigenic substances that will safeguard and secure its grips within the system of the host, while the immune system of the host in an attempt to fight the parasite releases several antibodies. Due to this cyclical and sustained multiplicative nature, the antigenic substance released by the parasite may hyper stimulate and/or weaken the defence

mechanism of the host (Bouree *et al*., 1994). Clinical presentations of malaria depend on the severity of the attack and the two types of presentation identified are:

- Benign or uncomplicated malaria: the attack is acute or mild and only become severe if left untreated or if treatment is delayed or improperly administered (Bouree *et al.*, 1994; Bouree, 1997). The classical manifestation include fever, headaches, pains, GIT effect and yellowing of the eye (jaundice which may be due to obstruction, excessive haemolysis of RBCs, infective damage of the liver cells or bile statis).

- Cerebral or complicated malaria which results exclusively from *P. falciparum* with high fatality rate, hyper parasitaemia, pyrexia and with CNS involvement ranging from convulsion to coma probably due to electrolyte imbalance. It also affects the kidney and liver functions, these consequences are more severe in children (White and Pukrittayakamee, 1993; Bouree *et al.*, 1994).

2.1.2.3 *Control and prevention of malaria*

The scourge of malaria is increasingly becoming more demanding and challenging not only to the medical professionals, health services, scientific and research world, but also to the governments (Murphy and Breman, 2001). The main global control strategies or approaches now being used to tackle malaria include:

i. Early diagnosis and prompt effective treatment of cases.

ii. Chemoprophylaxis and chemotherapy in susceptible groups.

iii. Vector control.

iv. Reduction of man-vector contact.

v. Surveillance, Information, Education and Communication.

vi. Vaccine and Research (Santiso, 1997; Lindsay *et al.*, 1998; Murphy and Breman, 2001).

Chemoprophylatic drugs do not prevent the attack of malaria, but merely reduce the risk of the severity and fatality. Chemoprophylatic treatment is needful only for individuals in the high-risk group: children, pregnant women, non-immune immigrants or travellers (Mutabingwa *et al*, 1993; Luzzi and Peto, 1993). However, Individuals residing in endemic areas do not require antimalarial chemoprophylaxis because they tend to acquire partial or natural immunity from the frequent or repeated exposures to the infection (WHO, 1998). Prevention is aimed at keeping the parasite completely away from the host so as to reduce morbidity and intensity of transmission. According to Greenwood (1997), reduction in human-mosquito contact consists of the use of appropriate prophylactic measures such as mosquito repellents, insecticide treated nets, pyrethrum house spraying and anti-mosquito fumigants. Other measures are practice of good sanitation, adequate hygiene, and adequate participation in public health education, vaccines and research. Surveillance is also necessary as it helps to promptly alert the authority of any risk of return of transmission.

2.2 Drug Treatment of Malaria

2.2.1 Importance of malaria chemotherapy

The essence of malaria chemotherapy is to prevent mortality, reduce morbidity and socio-economic loss in man-hours. Rational malaria chemotherapy consists of timely supportive treatment (symptomatic care) often in the form of:

i. Ensuring adequate hydration of the patient

ii. Prompt reduction of fever with antipyretics

iii. Reduction of headaches, body and joint pains using analgesics

iv. Prevention of vomiting with antiemetic.

v. Adequate feeding of the patient to maintain caloric requirements, prompt specific treatment of both the underlying disease complications and the malarial parasite (Bouree, 1997; Marsh, 1999).

Malaria treatment varies with the clinical spectrum (Marsh, 1999), but most malaria parasites are sensitive to the widely used antimalarials irrespective of the geographical origin of the parasite or patient (Skinner *et al*, 1996). However, there has never been a single ideal drug to combat malaria infection since all the available antimalarials are being threatened by the emergence of parasite resistance. Commonly used chemotherapeutic agents may be classified chemically or according to their point of attack on the malaria parasite life cycle e.g. primaquine acts on the gametocytes and chloroquine acts on the schizonts. Antimalarial drug combination therapy is simply defined as the simultaneous administration of two or more blood schizonticidal antimalarial drugs (co-formulated or co-administered) with independent modes of action and different biochemical targets in the parasite (malariasite, 2009). A relatively weak antimalarial is sometimes combined with a more active one as separate products given together or sequentially to enhance the action of the more active product. In some cases, an effective rapidly-acting antimalarial with a short duration of action is given with an equally effective, more slowly acting product with a long duration of action in order to reduce duration of administration of the short acting drug or to prevent recrudescence which commonly occurs when the short acting drug is given alone e.g. artemether and sulphadoxine- pyrimethamine (Yang and Liew, 1993; Barradell and Fitton, 1995). Combination therapy is one method of overcoming the global challenge of drug resistant *P. falciparum* (Yang and Liew, 1993; Taylor *et al*., 2001). Combination therapy is already a standard practice in many

Asian and Latin American countries and combinations that include artemisinin derivatives are now first-line treatment in much of S.E. Asia (Looareesuwan *et al*., 1992; Werndorfer., 1994). The most important factor currently affecting available antimalarial drug is resistance (Salako, 1998).

2.2.2 Antimalarial drugs

Antimalarial drugs are therapeutic agents for the prophylaxis and/or treatment of malaria. The rationale for the development and uses of each agent is considered in terms of the biology of malaria infecting parasites. Therapeutic agents used as antimalarials include: artemisinin, chloroquine, quinine, quinidine, mefloquine, halofantrine. Pyrimethamine and the antibiotics; sulfones, sulphonamides, and tetracycline are also used as antimalarials, though they are slow acting and less effective than the former groups (Katzung and Masters, 2011). Thus, antimalarial can be classified based on their pharmacological activity and chemical structure.

2.2.2.1 Classification based on antimalarial activity

- Tissue *schizonticides for causal prophylaxis*: These drugs act on the primary tissue forms of the plasmodia which grow within the liver and initiate the erythrocytic stage. By blocking this stage, further development of the infection can be theoretically prevented. Pyrimethamine and primaquine have this activity. However, since it is impossible to predict the infection before clinical symptoms begins this mode of therapy is more theoretical than practical.
- *Tissue schizonticides for preventing relapse*: These drugs act on the hypnozoites of *P. vivax* and *P. ovale* in the liver that cause relapse of

symptoms on reactivation. Primaquine is the prototype drug and pyrimethamine also has such activity.

- *Blood schizonticides*: These drugs act on the blood forms of the parasite and thereby terminate clinical attacks of malaria. They are the most important class of antimalarial drugs e.g. chloroquine, quinine, mefloquine, halofantrine, pyrimethamine, sulfadoxine, sulfones, tetracycline etc.
- *Gametocides*: This class destroys the sexual forms of the parasite in the blood. Chloroquine and quinine have gametocidal activity against *P. vivax* and *P. malariae*, but not against *P. falciparum*. Primaquine has gametocidal activity against all plasmodia, including *P. falciparum*.
- *Sporontocides*: These drugs prevent the development of oocytes in the mosquito and thus prevent the transmission. Primaquine and chloroguanide have this action.

2.2.2.2 Classification based on structure

a. *Aryl amino alcohols:* This class consists of Quinine, quinidine (cinchona alkaloids), mefloquine, and halofantrine.
b. *4-aminoquinolines:* This class consists of chloroquine, amodiaquine.
c. *Folate synthesis inhibitors:* These are type 1 - competitive inhibitors of dihydropteroate synthase - sulphones, sulphonamides, and type 2 - inhibit dihydrofolate reductase - biguanides like proguanil and chlorproguanil; diaminopyrimidine like pyrimethamine.
d. *8-aminoquinolines:* e.g. Primaquine
e. *Antimicrobials:* This class consists of tetracycline, doxycycline, clindamycin and azithromycin.

f. *Peroxides:* This class consists of artemisinin (Qinghaosu) derivatives - artemether, arteether, artesunate, artelinic acid.

g. *Naphthoquinones:* Atovaquone belongs to this class.

2.3 Malaria Chemotherapy in Pregnancy

2.3.1 Malaria in pregnancy

Malaria is a common infection worldwide and a major health problem particularly during pregnancy (Archibald, 1956; Brabin, 1983). Cerebral malaria is a common complication of severe *P. falciparum* infection with high mortality rate. Pregnant women are at high risk of becoming infected as well as children in their early years of life (Santiso, 1997). Malaria is a recognized cause of perinatal/maternal illnesses and complications that occur during pregnancy (Mvondo *et al.,* 1992), including spontaneous abortions and / or miscarriages, premature delivery, congenital malaria in the new-born, intrauterine growth retardation or low birth weight babies, foetal and maternal death (Hoffman *et al.*; 1992; Singh *et al.*, 1999; Jimoh, 2003). It is the leading cause of indirect obstetric deaths that is often mistaken for other common illnesses (Klufio, 1992). The severity of malaria during pregnancy varies according to the initial immunity of the pregnant woman, but generally, immunity to malaria regresses during pregnancy leading to the development of some latent malaria and /or the severe form of the illness (Klufio, 1992; Ismail *et al*., 2000). Thus, the disease is particularly dangerous for pregnant women in whom the benign malaria may result to violent prenatal and maternal illness or death. The risk is greater during the first and second pregnancies and occurs mostly towards the first trimester period irrespective of acquired immunity (Fried and Duffy, 1996). The risk of caesarean section, still births and neonatal mortality, has been shown to be high in women who had attacks in

the first and second trimesters of pregnancy (Taha and Gray, 1993). Malaria in pregnancy is usually asymptomatic and often associated with a negative peripheral-blood film (Shulman, 1999). While maternal parasitaemia leads directly to placental infection, it indirectly affects foetal growth by causing maternal haemolysis and anaemia (McGregor, 1984). Accumulation of infected RBCs in the placenta leads to impairment of materno-foetal exchange of oxygen and nutrients resulting in severe acute respiratory distress syndrome (Matteelli *et al.*, 1997). Malaria can cause maternal anaemia which can be the major cause of maternal mortality especially during the first pregnancy (Santiso, 1997; Shulman, 1999). Maternal haemoglobin concentration has been found to be significantly reduced in malaria-infected women and reduced haemoglobin was the main determinant of pre-term delivery (Allen *et al.*, 1998). Foetal anaemia is also frequently seen in malaria infection during pregnancy due to the deficiency of and / or iron transfer from the mother resulting in retarded intra-uterine growth and low birth weight (Santiso, 1997). The *utero* transmission of plasmodium from the mother to the infant may also result in congenital malaria (Subramanian *et al*., 1992; Fischer, 1997). Congenital malaria is the presence of plasmodium parasite in the erythrocytes of new-borns less than seven days old which can be diagnosed using cord blood (Bouree *et al.*, 1994; Balaka *et al*., 2000; Ismail *et al*., 2000) and three types are distinguished based on severity:

i. Simple congenital malaria with only a spontaneous and diminishing parasitaemia.

ii. Symptomatic congenital malaria: - this is rare but fatal; it is characterized by foetal distress, hepatomegaly, jaundice and infantile pallor. It causes poor ossification, low birth weight and growth retardation.

iii. Perinatal malaria - this is the type contracted at the time of birth and which takes several weeks to manifest (Bouree *et al.,* 1994).

2.3.2 Malaria in early childhood

The new born Infants do not acquire their own antibodies until about 6 months of age (Wagner *et al.,* 1998), but children under 6 months of life are significantly protected by the passive immunity of maternal antibodies and so may not contract clinical malaria or the manifestations may be mild with low-grade parasitaemia (McGuinness *et al.,* 1998; Wagner *et al.,* 1998). Thus, lactation is therefore very important within the first 6 months of birth to protect a child from malaria attacks amongst other factors. Children between 6-12 months of age are the easy targets or victims of malaria especially when the maternal antibodies are lacking or insufficient to protect them (McGuinness *et al.,* 1998). It has been established that by the age of 7-10 years, children who have survived growing up in malaria endemic areas will have acquired immunity naturally through repeated infections, and that such children do build up enough antibodies that enable them to resist malaria's consequences (WHO, 1998).

2.3.3 Clinical status of antimalarials in pregnancy

The first trimester of pregnancy carries the highest risk of foetal adverse reactions and some women are exposed to medicines during this period unaware that they are pregnant or do not declare their pregnancy (Ward *et al.,* 2007). Recently, studies have described drug exposure prevalence of 86-97% with an average of 2.9 – 4.2 drugs per woman (Beyens *et al*., 2003), In practice, clinicians make decisions to use drugs in pregnancy on the basis of their pragmatic assessment of the risk-benefit ratio to the mother and the unborn child. A good example of such a process has been the

acceptance of chloroquine as a drug suitable for use in all three trimesters of pregnancy before its fall from favour because of parasite resistance (Ward *et al.*, 2007). Host defences against malaria are impaired in pregnancy and pregnancy itself creates huge physiological changes which include: increased volume of distribution, reduced gut motility, increased renal blood flow, hormonal changes and increased protein binding all of which can alter drug disposition and metabolism (Ward *et al.*, 2007). Unfortunately, when pharmacokinetic studies are done they usually only include adult men. The number of pregnant women treated with antimalarials who have been included in drug pharmacokinetics studies worldwide is less than 100 (Nosten *et al.,* 2006). Incorrect dosing could result in maternal / foetal toxic effects, therapeutic drug failures and the large-scale deployment of intermittent preventive treatment increases the risk of drug resistance (Ward *et al.,* 2007). There are few pharmacokinetic and toxicity studies of antimalarials during pregnancy (Ward *et al.,* 2007). The plasma concentrations of the active metabolite of artemether (dihydroartemisinin) are lower in pregnant adult compared to non-pregnant adults (Noston *et al.,* 2000). Chloroquine readily crosses the placenta in human beings (Akintowa *et al.,* 1983). Sulphadoxine-pyrimethamine has been used extensively in pregnancy for intermittent preventive therapy strategies on the assumption that doses in non-pregnant adult are correct for pregnant woman. There are no pharmacokinetic data on the efficacy of sulphadoxine-pyrimethamine when used for case management in pregnancy (Ward *et al.,* 2007). Preclinical studies indicate embryo toxic effects including cleft palate in rat pups (Chung *et al*., 1993). Although, quinine has been used historically as an abortifacient, use of the drug in pregnancy is generally thought safe. A review of the clinical data on quinine use in pregnancy concluded that there was no evidence of poor birth outcomes in several hundred women treated with

quinine during pregnancy including almost 400 treated in the first trimester (Nosten *et al.,* 2006).

Indiscriminate use of medicines in pregnancy is not recommended because of the risk of adverse reactions in the mother and foetus and the possibility of irreversible effects. The decision to give drugs to pregnant woman must be made based on potential benefits outweighing the potential risk to the foetus. Causality assessment is difficult in pregnant woman because some adverse reactions can only be identified after delivery (Ward *et al.,* 2007). But, untreated malaria poses a far greater risk than treatment.

2.4 Pharmacology of Some Antimalarial Drugs

2.4.1 Quinine

Quinine is a cinchona alkaloid that belongs to the aryl amino alcohol. It is an extremely basic compound and is therefore, always presented as a salt (Yakoub *et al.,* 1995). Various preparations exist; including the hydrochloride, dihydrochloride, sulphate, bisulphate, and gluconate salts of which the dihydrochloride is the most widely used. The discovery of quinine is considered the most serendipitous medical discovery of the 17th century and malaria treatment with quinine marked the first successful use of a chemical compound to treat an infectious disease. Quinine and other cinchona alkaloids including quinidine, cinchonine and cinchonidine are all effective against malaria, all four alkaloids found to be comparable and with cure rates of >98% (Yakoub *et al.,* 1995). Quinine remained the mainstay of malaria treatment until the 1920s when more effective synthetic antimalarial became available. With increasing resistance to chloroquine, quinine again played a key significant role in the management of malaria.

2.4.1.1 Pharmacokinetics of quinine

Quinine has rapid schizonticidal action against intra-erythrocytic malaria parasites. It is also gametocidal for *P. vivax* and *P. malariae*, but not for *P. falciparum*. Quinine is rapidly absorbed both orally and parenterally, reaching peak concentrations within 1-3 hours (Salako and Sowunmi, 1992). It is distributed throughout the body fluids and is highly protein bound mainly to alpha-1 acid glycoprotein. Quinine readily crosses the placental barrier and is also found in cerebral spinal fluid. Excretion is rapid with 80% of the administered drug eliminated by hepatic biotransformation and the remaining 20% is excreted unchanged by the kidney (White, 1996, Esamai *et al.*, 2000). Quinine is used for the treatment of severe illness due to chloroquine and multi-resistant strains of *P. falciparum* in which intravenous quinine is mandatory and preferable (Kager and Wetsteyn, 1996). It is also used for simple episodes of fever in many tropical zones (Bouree, 1997). Quinine is used in combination with primaquine (the only commercially available drug against hypnozoites) for the radical cure of relapse in *vivax* malaria (Claessen *et al*., 1998; Tracy and Webster Jr., 2001; Nicolas *et al*., 2000). Quinine is used in combination with tetracycline or doxycycline in resistance cases as previously proposed (Fontanet *et al*., 1993; Kager and Wetsteyn, 1996).

2.4.1.2 Adverse effect of quinine

The side effects commonly seen at therapeutic concentrations are referred to as cinchonism with mild forms including tinnitus, slight impairment of hearing, headache and nausea. Impairment of hearing is usually concentration-dependent and reversible (Karlsson, 1990). Hypoglycaemia was reported in up to 32% of patient receiving quinine therapy (Okitolonda *et al.*, 1987; White, 1996), especially in pregnancy (Looareesuwan *et al.*, 1985).

2.4.2 Chloroquine

Chloroquine is a member of 4-aminoquinoline drug used in the treatment and prevention of malaria infection. It has a rapid schizonticidal action against all forms of malaria parasites and thus used both for controlling acute attack and for complete cure of *falciparum* malaria (Bloland *et al.,* 1998). Chloroquine is reported to be safe, cheap, more potent and less toxic, has short treatment course, effective against sensitive malaria parasites and better tolerated than quinine (the next alternative). It remains the treatment of choice for malaria caused by *P. vivax, P. ovale* and *P. malariae* but it is no longer useful for *P. falciparum* ma1aria due to resistant cases in Africa. Though rapid transfer of chloroquine into the placenta has been noted (Akintonwa *et al.,* 1983), there has been no report of an association between chloroquine administered during pregnancy as an antimalarial agent and foetal abnormalities (Subramanian *et al.,* 1992; Luzzi and Peto, 1993). However, failure to clear *P. falciparum* parasitaemia after chloroquine administration is now very common in pregnant women (Singh *et al.,* 1999), both spontaneous abortions and foetal anomalies were observed with the use of chloroquine-proguanil (Philips-Howard *et al.,* 1998). Chloroquine is valuable in the treatment of extraintestinal amoebiasis and giardiasis and produces symptomatic improvement in babesia infection (Tracy and Webster Jr., 2001). It has some anti-inflammatory properties and thus, may be used in rheumatoid arthritis and discord lupus erythematosus (Veinot *et al*., 1998; Onigbogi *et al*., 2000).

2.4.2.1 Pharmacokinetics of chloroquine

Chloroquine is rapidly absorbed following oral administration and highly concentrated in the RBC, liver, kidney, lung, leukocytes and melanin containing

tissues e.g. skin, pineal gland. Chloroquine has a high volume of distribution, as it diffuses into the body's adipose tissue, penetrates the CNS and transverse the placenta. Chloroquine resistance is associated with reduced sensitivity to other drugs such as quinine and amodiaquine.

2.4.2.2 Adverse effects of chloroquine

The toxic effects from the recommended dosage are mild and include: transient headache, visual disturbances sometimes leading to retinopathy (partly due to its preferential accumulation within the retina), gastrointestinal tract upset, and pruritus, skin lichenoid (mild skin eruption), slight weight loss and ototoxicity (Veinot *et al*., 1998; Onigbogi *et al*., 2000). A range of serious CNS effects had been documented during chloroquine therapy but the incidence is unclear (Philip-Howard and terKuile, 1995).

2.4.3 Sulphadoxine/Pyrimethamine

Sulphadoxine/pyrimethamine is a folic acid antagonist antimalarial agent. Each tablet containing 500mg N1-(5,6-dimethoxy-4-pyrimidinyl) sulfanilamide(sulfadoxine) and 25mg 2,4 diamino 5 (p chlorophenyl) 6 ethylpyrimidine (pyrimethamine). Sulfadoxie inhibits the activity of dihydropteroate synthase whereas pyrimethamine inhibits dihydrofolate reductase. Sulfadoxine/pyrimethamine is active against the asexual erythrocytic stages of *Plasmodium falciparum*. Sulphadoxine/pyrimethamine (SP) may also be effective against strains of *P. falciparum* resistant to chloroquine. *P. falciparum* malaria that is clinically resistant to SP occurs frequently in parts of Southeast Asia and South America and is also prevalent in East and Central Africa. Therefore, SP should be used with caution in these areas. Likewise,

Sulphadoxine/pyrimethamine may not be effective for treatment (or prophylaxis) of recrudescent malaria that develops after prior therapy with SP.

2.4.3.1 Pharmacokinetics of sulphadoxine / pyrimethamine

After oral administration of 1 tablet, peak plasma levels for pyrimethamine are approximately 0.2mg/L; and for sulphadoxine, 60 mg/L attained after about 4 hours. The volume of distribution for sulphadoxine and pyrimethamine is 0.14 L/kg and 2.3 L/kg, respectively. Plasma protein binding is about 90% for both pyrimethamine and sulphadoxine. Both pyrimethamine and sulphadoxine cross the placental barrier and are excreted into breast milk. About 5% of sulphadoxine appears in the plasma as acetylated metabolite and about 2 to 3% as the glucuronide. Pyrimethamine is transformed to several unidentified metabolites. A relatively long elimination half-life is characteristic of both components. Both pyrimethamine and sulphadoxine are eliminated mainly via the kidneys and in patients with renal insufficiency, delayed elimination of the components of pyrimethamine and sulphadoxine must be anticipated.

2.4.3.2 Adverse effects of sulphadoxine / pyrimethamine

Testicular changes have been observed in rats treated with 105 mg/kg/day of SP and with 15mg/kg/day pyrimethamine alone. Fertility of male rats and the ability of male or female rats to mate were not adversely affected at dosages of up to 210 mg/kg/day of SP. The pregnancy rate of female rats was not affected following their treatment with 10.5 mg/kg/day, but was significantly reduced at dosages of 31.5 mg/kg/day or higher, a dosage approximately 30 times the weekly human prophylactic dose. SP has been shown to be teratogenic in rats when given in weekly doses approximately 12 times the weekly human prophylactic dose. Teratology studies with SP (1:20) in rats

showed the minimum oral teratogenic dose to be approximately 0.9 mg/kg pyrimethamine plus 18 mg/kg sulphadoxine, there are no adequate and well-controlled studies in pregnant women. However, due to the teratogenic effects shown in animals and because pyrimethamine plus sulphadoxine may interfere with folic acid metabolism as well as report of kernicterus (in new-borns) due to bilirubin deposit in the brain at the third trimester of pregnancy. SP therapy should be used during pregnancy only if the potential benefit justifies the potential risk to the foetus. Women of childbearing potential who are traveling to areas where malaria is endemic should be warned against becoming pregnant and should be advised to practice contraception during prophylaxis with SP and three months after the last dose.

2.4.4 Artemether

Artemisinin derivatives are a relatively new group of drugs with antimalarial properties and presently the most rapidly acting and effective of all available antimalarial. They offer a genuine prospect of reducing mortality from malaria in the tropics (Karbwang *et al*., 1992, White and Pukrittayakamee, 1993). It produces more and rapid resolutions of fever and parasitaemia than all known antimalarial agents. Oral dihydroartemisinin has been introduced recently but there is considerable less data on this compound.

Qinghaosu is a prototype of the natural bridged endoperoxide antimalarial discovered as a major advance in the search for new antimalarial against multi-drug resistant strains of malaria parasite (Shukla *et al*., 1995, Skinner *et al*., 1996).

2.4.4.1 Pharmacokinetics of artemether

Artemisinin is poorly soluble in water but dissolves readily in other solvents. One of the several attempts made to improve the stability and solubility of artemisinin in both the aqueous and lipophilic milieu was the reduction of the lactone to a lactol with sodium borohydride (Lin *et al*., 1987; 1989; 1990). The resultant product dihydroartemisinin retained the potency of the parent compound but with improved water solubility. Pharmacological studies have shown artemether to be effective in the treatment of human cerebral malaria (Myint *et al*., 1989; Shwe *et al*., 1989) and uncomplicated falciparum malaria (Naing *et al*., 1988). Artemisinin drugs bind selectively to *P. falciparum*-infected erythrocytes to increase their endogenous level of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) dependent production of active oxygen species such as O_2 and H_2O_2 (Gu *et al*., 1984; Krungkrai and Yuthavong, 1987; Leskovac and Peggins, 1992). This leads to the reduction of the unsaturated fatty acid content of the plasmodia parasite (lipid peroxidation) resulting in oxidative stress and subsequent killing of the parasites (Pan *et al*., 1989). Plasmodia pigments (haemozoin) have a very strong affinity for endoperoxide-bridge and bind to dihydroartemisinin, the principal biological active metabolite of artemisinin drugs to form a haem complex. This haem complex once formed catalyses the release and oxidation of some free radicals or electrophilic intermediates of the malaria proteins such as protein thiol and amine. This alkylation or poisoning of one or more of the essential malaria proteins results in inhibition of the cytochrome oxidase and/or the proteolytic activity of its food vacuole in which it digests the ingested cytosol or haemoglobin of its host (Zhao *et al*., 1986, Foley and Tilley, 1998). Thus, the inability to further degrade haemoglobin and to polymerize the degraded toxic haem into insoluble haemozoin results in killing of the parasite

(Meshnick, 1998). Animal studies showed that the fat-soluble derivatives artemether and arteether are approximately twice as active as other derivatives *in-vivo*, but the water-soluble dihydroartemisinin and artesunate possess superior *in-vitro* activity to artemisinin, arteether and artemether against *P. falciparum* (Bunnag *et al*., 1996; de Vries and Dien 1996). Biotransformation into the active metabolite dihydroartemisinin was seen to occur rapidly and almost immediately as the glucuronide conjugate of the phase-1 metabolite was detected in rat plasma 15 minutes post injection (Ramu and Baker, 1997). The mean elimination half-life of artemether is 2-5 hours and elimination via biliary excretion of conjugated dihydroartemisinin was also reported (Leo *et al*., 1997). Liver has been shown to be the chief site of artemisinin inactivation (Leskovac and Theoharides, 1991) and a first pass effect has been found to occur with the oral formulations of the drug (Navaratnam *et al*., 2000).

2.4.4.2 Adverse effect of artemether

Generally, artemether was found to be well tolerated when used both as a single drug regimen and in combination therapy with mefloquine (Price *et al*., 1999; Wilairatna *et al*., 2000). From the results of series of clinical safety reviews, there has not been any report of a serious or significant toxicity associated with the usual clinical doses (Philips-Howard and ter-Kuile, 1995) and according to Zhao (1986) conventional doses of this drugs show relatively high selectivity as antimalarial. The adverse reactions were only associated with higher doses; but mild and transient cases include, acute nausea, vomiting, anorexia, dizziness, pain at the injection site, palpitation, abdominal pain, diarrhoea, occasional slight decrease (neutropenia, reticulocytopenia)

and increase (eosinophil) in the number of blood components. Slow rate of heart contraction (transient bradycardia) which often leads to prolonged QT interval and bizarre T-wave, hypoglycaemia, elevated liver transaminases (AST and ALT) and few cases of neuropsychiatric episode during concomitant treatment with mefloquine (Bunnag *et al*, 1993; Brewer *et al.*, 1994; Barradell and Fitton, 1995; Ribeiro and Olliaro, 1998; Price *et al*., 1999; Nabangchang *et al.*, 2000). In higher than the normal doses, artemisinin derivatives were found to be toxic to the brain, bone marrow, kidney and liver of experimental animals (Davidson, 1994). The true safety and / or efficacy of these drugs in pregnant women have not been well defined or established (McGready and Nosten, 1999). Artemisinin drugs have also been shown to possess the potential of by-passing the quinoline resistance machinery of the plasmodia parasites which normally causes sub-lethal accumulation of antimalarial drugs in resistant strains. This may explain the efficacy of these drugs on resistant strains of *P. falciparum* (Pandey *et al*., 1999).

2.5 The Oestrous Cycle

2.5.1 Rat oestrous cycle

The average rat life span is approximately 2-3 years depending on the stock/strain, sex, health/disease status and genetic background of animal. By 2 months of age, young female rats are reproducibly mature and exhibit oestrous cycle every 4 to 5 days (Kohn and Clifford, 2002). After a year, rat cycle length increases slightly and lasts about 6 days till the end of the reproductive life span (Lu *et al.*, 1994). The mean oestrous cycle is 4.4 days (Abiodun *et al.,* 2012). Oestrous cycle starts after puberty in sexually matured female rats and are interrupted by anoestrous phases or pregnancy.

The oestrous cycle comprises of the recurring physiologic changes that are induced by reproductive female sex steroid, whose levels vary in intact female rats depending on the oestrous cycle stage and length, reproductive status and age (Sylvia *et al.,* 2005). The cycle is made up of four different stages namely: proestrus, oestrus, metoestrus and dioestrus stage (Long and Evans, 1922; Freeman, 1988), unless interrupted by pregnancy, pseudo pregnancy or anoestrous. The characterization of each phase is based on the proportion among three types of cells observed in the vaginal smear which are; epithetical cells, cornified cells and the leukocytes. The crucial event in the reproductive activity of the female is the ovulation; it is brought about by the circulating levels of ovarian oestrogens. The level of oestrogen differs with different stages of the oestrous cycle, the highest levels of estradiol occur during early proestrus with values decreasing to their lowest levels during oestrus and metoestrus. With respect to progesterone, the highest levels have been observed during late proestrus (Smith. *et al.,* 1975; Spornitz. *et al.,* 1994) and early oestrus and lowest levels during metoestrus to dioestrus (Butcher *et al.,* 1974, Nequin *et al.,* 1979).

Oestrous cycle is also known to be disrupted by genetics, nutritional and endocrine factors, acting at different levels of hypothalamic-pituitary axis or at ovarian level to inhibit ovulation (Astwood, 1939).

2.5.2 Factors affecting the oestrous cycle

- *Environmental factors***:** Rodent oestrous cycles are very sensitive to changes in the light: dark cycle. 12:12 hours ratio is the standard, a change in this standard could lead to different ratio in the number of 4 and 5 days cycles. Longer periods of light within 24 hours may result in an irregular,

lengthened cycles and continuous lighting will effectively cease cycling and an extended period of vaginal oestrus will be observed.

- *Smearing technique*: The two most commonly used methods are the pipette vagina lavage and swab technique. The pipette technique has low risk of pseudo pregnancy. If the pipette is inserted anteriorly or too deep in the vaginal cavity this will cause cervical stimulation and eventually pseudo pregnancy which extends the cycle to about 12 – 14 days. Swab technique has a higher incident of pseudo pregnancy, it shows fewer leucocytes at metoestrus and dioestrus and a low but variable incidence of cornified cells at all stages of the cycle thus making stage identification false or difficult.

2.5.3 Different stages of oestrous cycle and cell types

There are basically four stages of oestrous cycle namely proestrus, oestrus, metoestrus and dioestrus. The three main cell types seen in vaginal smear from rat can be described morphologically as:

- *Cornified (keratinized) cells*: they are large, angular and irregularly shaped mostly non-nucleated when mature (as seen at oestrus).
- *Epithelial cells*: not quite as large as the cornified cells and much more rounded in shape. Those seen at proestrus are mostly nucleated and with a granular appearance while those seen as metoestrus tend to be non-nucleated and less granular.
- *Leucocytes*: they are very small, round and the nucleus is not usually evident at the low magnification used.

The proportion between these cell types at each stage permits the classification of the oestrous cycle (OECD).

2.5.3.1 *Proestrus stage*

Proestrous smears (Figure 2.2a) are characterized by rounded, usually nucleated, epithelial cells generally in low to moderate (occasionally high) numbers. It is a short stage, normally occurring the day before oestrus. But often not seen until mid-morning so may appear to be missing if smears are taken early in the morning. Quite often, it is seen as a transitional smear in combination with preceding day of dioestrus. Some epithelial cells may show early stages of cornification i.e. becoming larger and more irregular in shape. A low incidence and degree of cornification at proestrus is normal but if it is more marked then the smear may be recorded as proestrus-oestrus (PE), although, still regarded as proestrus for cycle staging purposes, provided the smear is seen the day before oestrus or three days after the last oestrus. It is characterized by functional involution of corpora lutea. Some follicles grow rapidly and the vaginal smear reveals entirely nucleated corpora epithelial cells. The vagina becomes dry and the uterus starts to become distended. The duration is approximately 12 hours (Lawson, 2001).

2.5.3.2. Oestrus stage

Oestrus smears (Figure 2.2b) consist entirely of cornified cells which are large, often non-nucleated and abundant forming clumps and sheets visible to the naked eye on swab smear slides and cell suspension. It is a long stage starting during the preceding dark phase and lasting until at least early afternoon and is normally seen every 4th day but can occur on two or more consecutive days (5-day cycle). This stage is referred to as the heat period (ovulation occurs) characterized by rapid maturation of multiple ovarian follicles, uterine enlargement and rapid proliferation of vaginal mucosa resulting in the exfoliation of cornified squamous cells. The oestrogen level at this stage of the cycle is high and the stage lasts 9-15 hours (Lawson, 2001).

2.5.3.3 Metoestrus

The metoestrus stage is intermediate between oestrus and dioestrus stage and occurs shortly after ovulation. Metoestrus smears (Figure 2.2c) reveal the presence of numerous leukocytes along with some cornified squamous cells and spotted epithelial cells. The leucocytes are often characteristically in contact with other cell types, forming small tightly packed clumps of cells and normally seen only on the day after oestrus. It can be a short phase appearing early and so may be "missed" resulting in two days of dioestrus. Occasionally, the metoestrus characteristics can carry over into the next day with a transitional metoestrus / dioestrus (MD) smear. The transition from oestrus to MD provides a marked change in smear appearance and is an essential reference point for the calculation of cycle lengths because it confirms that the ovulatory point has passed thereby demonstrating the completion of one cycle (oestrous) and beginning of the next cycle. In the very early phase of metoestrus, cornified and large epithelial cells predominate, with small numbers of leucocytes. The ovaries contain corpora lutea, the oestrogen level is low compared to the oestrus and proestrus stages and the vaginal wall becomes moist, while the uterus appears fleshy and pink. This stage normally last between 14-18 hours (Lawson, 2001).

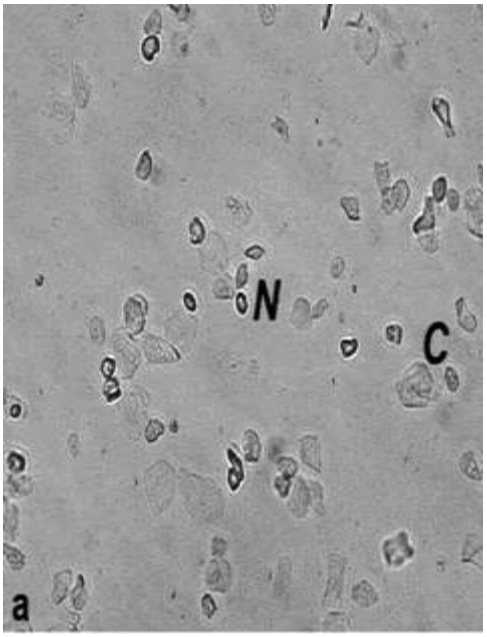
2.5.3.4. Dioestrus

Dioestrus smears (Figure 2.2d) consist mainly of leucocytes, but with quite variable numbers of epithelia and small cornified cells. The cell numbers are low to moderate and the general appearance is similar to metoestrus, but usually with far fewer cells and without the tightly packed clumps. Dioestrus can be the most difficult stage to recognize because of the variability in the numbers and ratios of the cells. It is the longest stage normally present throughout Day 2 (after metoestrus) but may also be

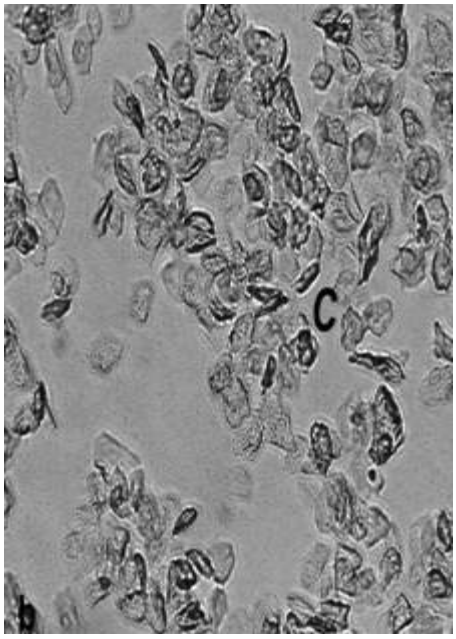
present on Day 1(after oestrus) and / or Day 3 (before oestrus). Dioestrus is also the 'resting' stage of the cycle and females that become pseudopregnant and stop cycling (acyclic) will show consecutive days of dioestrus, sometimes accomplished by swirls or strings of mucus (not seen in cycling dioestrus smears). The vaginal wall is moist and the uterus is in a resting stage. This stage last approximately 60-70 hours (Lawson, 2001).

Although, it is possible to easily recognize the majority of smears as typical examples of oestrus, metoestrus, dioestrus or proestrus (E, M, D or P respectively) or a transition between two of these stages. Some smears are difficult to classify especially with some types of treatment-related changes. Whilst a reasonable effort should be made to classify smears by a cycle stage, it is sometimes better to record just the cell types for a genuinely atypical smear rather than 'forcing' a particular stage classification onto a smear. Smear assessments can be recorded according to the following degrees of precision:

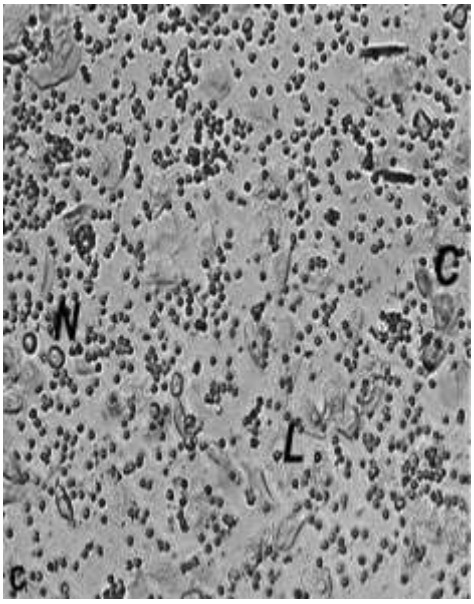
- Single Stage (E, M, D or P) – if within the normal range cell of appearance.
- Transitional stage (e.g. MD or DP) – if characteristic of both stages.
- Single or transitional stage with additional comment – if recognizable but not typical e.g. D + Corn (D but with more cornified cells than normal).
- Cell types only – if not sufficiently normal to classify as a stage.



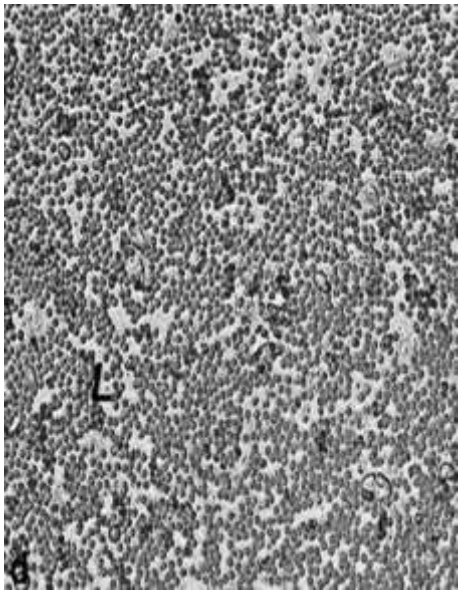
Proestrus



Oestrus



Metoestrus



Dioestrus

Figure 2.2 Photomicroscopic images of the different cell types seen in vaginal smear of rat. N= nucleated epithelial cell, C= cornified squamous cell and L= leucocyte Magnification X40
(Byers *et al.*, 2012)

2.5.4 Various methods of determining the oestrous cycle in rats

Changes occurring in the rat oestrous cycle are evident in the animal's physiology and anatomy (Byers *et al.,* 2012*)*. These changes can be detected using a variety of methods to determine the stages of oestrous cycle (Allen, 1922; Caligioni, 2009).

2.5.4.1 Techniques used in smearing:

The two most commonly used techniques are:-

- Lavage or washing with saline from a pipette
- Swab or cotton bud (moistened with saline).

Each of the above method is not without advantage or disadvantage in terms of expertise and time required to take the smear. The pipette method is most recommended because there is limited monitoring of cycle and risk of pseudo pregnancy is very low as the sample can be viewed and read almost immediately. Disposable pipette made of soft plastic with an internal tip bore of approximately 1.5 mm are recommended this will prevent cross contamination of the results from each animal. The disposable pipette should be inserted into the vagina posteriorly to prevent cervical stimulation and pseudo pregnancy during oestrus which will lengthen the cycle. The swab method unlike the pipette method shows fewer leucocytes at metoestrus and dioestrus and a low but variable incidence of cornified cells at all stages of the cycle. This makes the smear taken by this method somewhat more difficult to read compared with the pipette smear and it also has a high incidence of pseudo pregnancy thereby lengthening the oestrous cycle.

The pipette technique: A small amount of 0.9% normal saline is drawn up using a plastic pipette and the rat is held around the thorax ventral surface uppermost. The tip of the pipette is inserted gently into the entrance of the vagina posteriorly and the fluid flushed into the vagina and back up into the pipette two or three times by gently

squeezing and releasing the bulb of the pipette (but if after one flushing and the fluid looks cloudy further flushing is not necessary). The resulting cell suspension is expelled unto a microscopic glass slide and viewed immediately under the electronic microscope at a magnification of X40. The cells observed are read and the stage identified based on the proportion or degree of dominance of cell types present in the smear.

The swab technique: The cotton wool tip is moistened slightly by dipping into a jar of saline or distilled water and sharply flicking off any surplus. The rat is held around the thorax ventral surface uppermost, whilst providing lumber support as far as possible. Holding the swab stick close to the smearing tip gives maximum control and lightly gripping the tail with the same hand gives further support and minimizes movement of the rat. The tip of the swab stick is inserted carefully into the rat's vagina to a depth of approximately 1 cm, with a rotating action of the swab, vagina cell samples are collected and the swab stick is removed with the same rotating action. The aim is to achieve a single, brief "in and out" action so that any cervical stimulation is minimal and is insufficient to induce a pseudopregnant response. The tip of the swab is rolled gently onto a clean glass slide which is either read immediately or kept for later use with the relevant animal's number. The swab stick is discarded and a new one used for each animal. The different cell types seen are recorded and the stage of the oestrous identified based on the presence, absence or proportion of cell types present.

2.5.5 Oestrous cycle of human

In human the oestrus cycle is referred to as the menstrual cycle. The biological feedback mechanism involving the hypothalamus, anterior pituitary gland, ovaries, and endometrial lining of the uterus, control the average 28 days menstrual cycle. The

hypothalamus synthesizes gonadotropin releasing hormone (GnRH) and secretes the hormone in a pulse like manner with varying frequencies throughout the menstrual cycle. GnRH stimulates the anterior pituitary to produce and release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH acts on the ovaries to produce oestrogen and progesterone.

2.6 The Uterus

2.6.1 Anatomical and physiological consideration of the uterus

The uterine smooth muscle is characterized by a high degree of spontaneous electrical and contractile activity. Waves of decreased membrane potential with superimposed spike activity are associated with contraction. Cell to cell spread of excitation occurs, but electrical conduction is slow and decreasing in nature. Low-resistance contacts between cells (gap junctions) greatly facilitate the spread of excitation. The number of such junctions is regulated by steroid hormones and increases in the later stages of pregnancy. Increased frequency and duration of spike activity in "pacemaker" areas and more extensive spread of excitation are associated with increases in force of contraction. In most species (including the human female), the influx of Na^+ appears to play the primary role in depolarization. The amount of Ca^{2+} that crosses the plasma membrane during excitation is insufficient to cause contraction directly. It is sufficient, however, to trigger the release to much larger amounts of Ca^{2+} from the sarcoplasmic reticulum (Huszar and Roberts, 1982; Van Breemen and Saida, 1989). Hence, the availability of extracellular Ca^{2+} (or the presence of blockers of Ca^{2+}channels) strongly influences the response of uterine smooth muscle to various physiological and pharmacological stimuli.

Uterine smooth muscle is unusually susceptible to endocrine influence, especially that of the oestrogens. Thus, spontaneous activity, as well as responsiveness to neurogenic, hormonal and pharmacological stimulation, increase greatly at puberty and vary thereafter, with the ovulatory cycle (Tracy and Webster Jr., 2001). The uterus has parasympathetic and sympathetic innervation, both can elicit increased activity in the mature human uterus. Both α_1 and β_2-adrenergic receptors are clearly demonstrable in the myometrium of mammals. Other receptors present in the uterus are those of relaxin, which is a small peptide hormone produced by the corpus luteum and the placenta and which inhibits uterine contraction (Weiss, 1987). Excitatory receptors for oxytocin that have been demonstrated are prostaglandin E_2 and $F_{2\alpha}$ and, in some species, 5-hydroxytryptophan (5-HT). They are found to increase uterine contractile activity (Tracy and Webster Jr., 2001).

2.6.2 Drugs with effect on the uterus

Apart from the endocrinological status, contractile responses of smooth muscle are strongly influenced by variable factors such as period of gestation, the degree of stretch, and the region of the uterus under consideration. Thus, it is not surprising that there are many conflicting reports of the effects of drugs on this organ (Tracy and Webster Jr., 2001). Drugs that affect the uterine smooth muscles either stimulate or inhibit it causing contraction or relaxation and thus referred to as oxytocics or tocolytics respectively.

2.6.2.1 Oxytocics

These are agents that stimulate the uterine smooth muscle bringing about their contraction. There are several indications for, and contraindications to, the clinical use of agents that stimulate uterine contractions. The clearest indications are;-

- To induce or augment labour in selected individuals
- To control postpartum uterine atony and haemorrhage
- To cause uterine contraction after caesarean section or during other uterine surgery, and
- To induce therapeutic abortion (Tracy and Webster Jr., 2001).

Members of this class of drugs include:

2.6.2.1.1 Oxytocin

This is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus within neurons that are distinct from those that contain antidiuretic hormone (ADH) (Tracy and Webster Jr., 2001). Oxytocin has selective stimulatory effects on the smooth muscle of the uterus potent enough to suggest that the polypeptide serves a true hormonal function at this site. It elicits contraction of the fundus that is indistinguishable in amplitude, duration and frequency from those seen in late pregnancy and during spontaneous labour. Sensitivity of the uterus to oxytocin increases as pregnancy progresses and the number of receptors for oxytocin in the myometrium and decidua is markedly increased in later stages of pregnancy. Oxytocin is present in both the maternal and foetal circulation during late gestation (Forsling, 1979). The effects of oxytocin are highly dependent on the presence of oestrogen, and the immature uterus is quite resistant (Tracy and Webster Jr., 2001). Oxytocin have dual effects in the uterus, it regulates the contractile properties of

myometrial cells and elicits prostaglandin production by endometrial/decidual cells, and in animal models, these effects are mediated through two distinct receptor sub-types suggesting that oxytocin antagonists designed as tocolytics agents for blocking of preterm labour must block both the uterotonic and prostaglandin-releasing effects of oxytocin and thus must block both oxytocin receptor subtypes (Chard *et al.,* 1971). Apart from effect on the uterus, oxytocin has effects on the mammary gland (expulsion of milk), cardiovascular system (decrease blood pressure, vasodilatation) and renal system (antidiuretic effect) (Tracy and Webster Jr., 2001).Oxytocin also suppresses the action of adrenocorticotrophic hormone (ACTH) (Legros, *et al.,* 1984).

2.6.2.1.3 Ergot alkaloids

The ergot alkaloids were the first agents used to initiate or accelerate parturition but now oxytocin is used for that purpose and the ergot alkaloids are most often used for treatment of postpartum haemorrhage. Though, all the natural alkaloids have qualitatively the same effect on the uterus in terms of increasing its contractile activity, ergonovine is most active and also less toxic than ergotamine (Tracy and Webster Jr., 2001).

2.6.2.1.3 Prostaglandins

Prostaglandins can be considered to be local hormones since (with few exceptions) they exert their effects and are inactivated principally in the tissues or organs in which they are synthesized. Prostaglandins are available as I, E and F series. Those found most abundantly in the uterus and in the menstrual and amniotic fluid, are of the E and F series. Prostacyclin (PGI_2) is confined largely to the uterine, umbilical, and foetal vasculature. Prostaglandins currently used in obstetric practice include $PGE_{2\alpha}$, $PGF_{2\alpha}$ and the synthetic derivative 15-methyl $PGF_{2\alpha}$ (Tracy and Webster Jr., 2001).

The sensitivity of the uterus to prostaglandins increases as gestation progresses and during the last two trimesters of pregnancy. The administration of either $PGE_{2\alpha}$ or $PGF_{2\alpha}$ causes strong uterine contractions and can induce delivery of the foetus (Tracy and Webster Jr., 2001).The principal side effects of Prostaglandin are stimulatory action on the alimentary canal. Hence, antiemetic and antidiarrheal drugs are administered concurrently. Other side effects are transient pyrexia, hypertension and vasodilatation (Tracy and Webster Jr., 2001).

2.6.2.2 Tocolytics

These are medications that inhibit uterine smooth muscle activity and bringing about uterine relaxation. There are several indications for, and contraindications to, the clinical use of agents that inhibit uterine contractions. The clearest indications are:

- To delay or prevent premature parturition in selected individuals.
- To slow or arrest delivery for brief periods in order to undertake other therapeutic measures. The several classes of tocolytics with different mechanism of action include;

2.6.2.2.1 β_2 adrenergic agonists

β_2 adrenergic agonists e.g. terbutaline and salbutamol: this class is used for low risk preterm labour. They are contraindicated in diabetic patient because they cause marked hyperglycaemia which usually does not require treatment, but persistent hyperglycaemia (>200mg/dl) may result in reactive hypoglycaemia in the infant should parturition proceed (Tracy and Webster Jr., 2001).

2.6.2.2.2 Magnesium sulphate

Magnesium sulphate (myosin light chain inhibitor): this is frequently given during pregnancy to control eclamptic seizures and also used as a highly effective inhibitor of uterine activity. Magnesium sulfate is an attractive alternative when β_2- adrenergic agonists are contraindicated because of their limited side effect (Tracy and Webster Jr., 2001).

2.6.2.2.3 Calcium channel blockers

Calcium channel blockers (Nifedipine). This group are known to relax the myometrium in vitro and to inhibit markedly the amplitude of oxytocin-induced contractions. Nifedipine is the most commonly used Ca^{2+} channel blocker for the treatment of preterm labour.

2.6.2.2.4 NSAIDs

NSAIDs Prostaglandin synthesis inhibitors e.g. indomethacin. They act by inhibiting the cyclooxygenase enzyme necessary for the conversion of arachidonic acid to prostaglandins. The use of this group is with minimal maternal side effect. However, concerns over a number of foetal and neonatal side effects limits its use throughout pregnancy, of particular importance is the possibility of premature closure of the ductus arteriosus and the development of pulmonary hypertension (Tracy and Webster Jr., 2001). Prolonged administration may also result in the development of oligohydramnios (de Wit *et al.*, 1988).

2.6.2.2.5 Oxytocin antagonist

Oxytocin antagonist (Atosiban), this agent, works primarily by blocking the action of oxytocin at the cellular level (Stuebe, 2012). Goodwin *et al.* (1994) in a randomized, blind placebo-controlled trial noted that atosiban resulted in a more significant inhibition of preterm contractions than did placebo.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Location of study

The study was conducted at the Department of Pharmacology and Therapeutics Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

3.1.1 Experimental animals

Young non-pregnant female Wistar rats weighing between 100 g and 180 g and pregnant Wistar rats weighing between 140 g and 250 g were obtained from the Department's Animal House. The non-pregnant rats were used for the oestrous cycle study while the pregnant rats were used for uterine motility study. The animals were kept in well ventilated cages at room temperature of 27^{o}C and were fed with standard rodent feed with access to tap water *ad libitum*. Animal care and handling was done in accordance with the International Regulations for Animal Research (NIH, 1985) and as approved by Animal Ethical Committee of the Department of Pharmacology and Therapeutics.

3.1.2 Materials

- 1 ml syringe.
- 10 ml sample bottles.
- Aerator.
- Animal cages and drinkers.
- Beakers.
- Binocular digital microscope (Boeco^{R,} MB-300, Germany).
- Calcium chloride (Fisher scientific company (New Jersey, USA).

- Electric microscope (fisher science education (China).
- Electric weighing balance (champ II CH15R, Oheus Corporation. Pins Brook, NJ, USA.
- Glass slides.
- Glucose (BDH Chemicals ltd. Poole, England).
- Manual weighing balance (Avery. Birmingham, England).
- Marker/paper tape.
- Microdynamometer (Churchill instrument co. ltd. Perivale England).
- Needle and thread.
- Organ bath.
- Pair of scissor.
- Pestle and mortar.
- Plastic disposable pipette.
- Potassium chloride (BDH Chemicals ltd. Poole, England).
- Slide covers.
- Sodium bicarbonate (BDH Chemicals ltd. Poole, England).
- Sodium chloride (BDH Chemicals ltd. Poole, England).

3.1.3 Drugs and Diluents

- Artemether (Makar laboratories limited Gujarat, India).
- Chloroquine (Shreechem pharmaceutical pvt. Ltd. Mumbai, India).
- Quinine (Wuhan Grand pharmaceuticals group co. ltd. Wuhan, China).
- Sulphadoxine/pyrimethamine combination (Ipca laboratories ltd. Mumbai, India).
- Oxytocin (Green-life pharmaceutical Co. Ltd. Jiangsu province,

China.

- Normal saline (Dana Pharmaceuticals, Nigeria).
- 3% v/v Tween 80 (BDH Chemicals ltd. Poole, England).

3.2 Methods

3.2.1 Oestrous cycle determination and effect of antimalarial drugs

The vaginal pipette smear technique of Allen and Doisy (1923) was adopted for evaluating the cycling pattern of rats, after which the effect of the antimalarial drugs: artemether, chloroquine, quinine and sulphadoxine/pyrimethamine combination were investigated. Approximately 0.2 ml of normal saline was drawn up into a pipette tip, the rat was held around the thorax with ventral surface uppermost. The tip of the pipette was pushed gently into the entrance of the vagina and normal saline was flushed into the vagina and drawn back up into the pipette two to three times by gently squeezing and releasing the bulb of the pipette. A small amount of the cell suspension was then expelled onto a glass slide and immediately viewed under the electric microscope at magnification of X40.

Cells were identified as cornified (keratinized), epithelial or leucocytes; and based on their relative proportions, the stages were determined as proestrous, oestrous, metoestrous and dioestrous as previously described in 2.5.3. Normal cycling is usually of 4 or 5 days duration, but because the latter were few, only cycles of 4 days were used in further experiments. Normal cycling rats were grouped into four treatment groups and two control groups. Group 1 were treated with artemether 3.2 mg/kg body weight loading dose and 1.6 mg/kg body weight maintenance dose for 7 days. Group II were treated with chloroquine 2.5 mg/kg every 4 hours (until a maximum of 25 mg/kg has been reached). Group III were treated with quinine 20 mg/kg body weight

loading dose and 10 mg/ kg body weight maintenance dose 8 hourly for 7 days and Group IV were treated with 25 mg/1.25 mg/kg body weight once, all treatment were given *i.p*. Groups V and VI were administered 1 ml/kg of normal saline and 1 ml/kg 3% v/v tween 80 as control groups. Artemether was made up in 3% v/v tween 80 and normal saline was employed for vaginal lavage. The animals were smeared between 9:00 am - 11:00 am each day and the corresponding drug was administered. This procedure was repeated daily for the duration of treatment and for four cycles post treatment.

3.2.2 Isolated uterine tissue and effect of antimalarial drugs

The method of Holton (1948) was adopted for rat uterus contractility study after which the effect of the antimalarial drugs; artemether, chloroquine, quinine and sulphadoxine / pyrimethamine combination on the uterine smooth muscles of rats were investigated. De jalon solution (Appendix XIV) was freshly prepared in the morning of the experiment Pregnant rats were sacrificed with a blow on the head, the abdomen was opened and the uterus was removed and placed in a Petri dish containing freshly prepared De jalon solution. It was later suspended in a 10 ml organ bath containing De jalon solutions which was continuously gassed with 95% oxygen, 5% carbon dioxide and maintained at 37^{o}C. After 30 minutes equilibration time, drugs where then added to the organ bath starting with oxytocin to ascertain tissue viability. The tissue was challenged with artemether at organ bath concentrations of 16-1280 µg/ml**.** With each addition of the drug to the organ bath it was allowed contact with the tissue for 30 second after which it was washed three times using De jalon solution and then allowed a resting period of 15 minutes before the addition of the next

concentration of drug. Responses were recorded using microdynamometer. Similarly, chloroquine, quinine and sulphadoxine/pyrimethamine combination were tested.

3.3 Statistical Analyses

Data obtained for the effects of the antimalarial drugs were expressed as mean ± SEM and subjected to one way analysis of variance (ANOVA) followed by LSD post hoc test. The statistical software used was SPSS version 18. Results were regarded as significant at $P<0.05$ or lower.

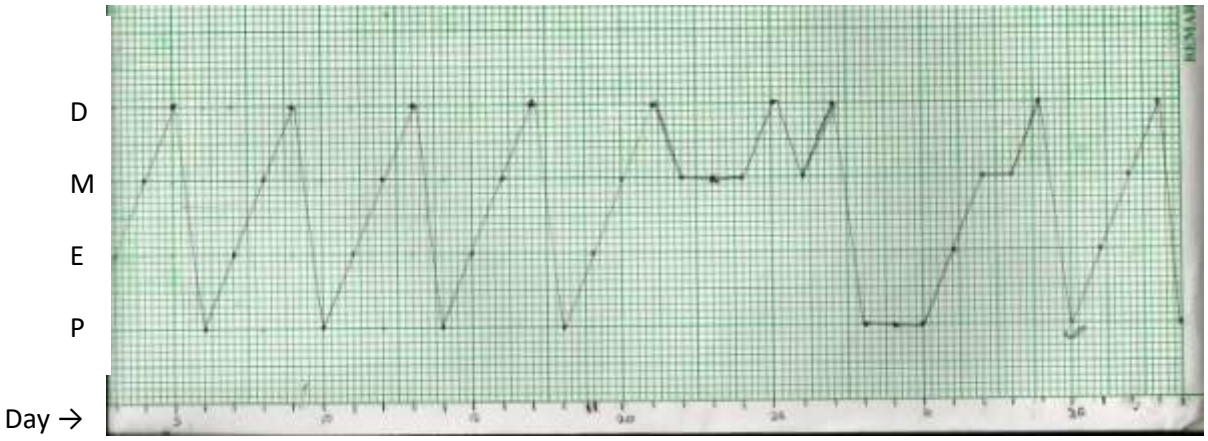
CHAPTER FOUR

4.0 RESULTS

4.1 Effects of the Antimalarial Drugs on oestrous cycle of female wistar rats

4.1.1 Artemether

The data obtained before, during and after artemether administration (32 mg/kg loading dose & 16 mg /kg maintenance dose) over a 39 day period showing the daily smear result (Appendix II) showed desynchronization of the oestrous cycle was observed (fig. 4.1) in which proestrus and oestrus stages are reduced ($p<0.05$) and metoestrus is increased ($p<0.05$) (fig. 4.2 and appendix III).



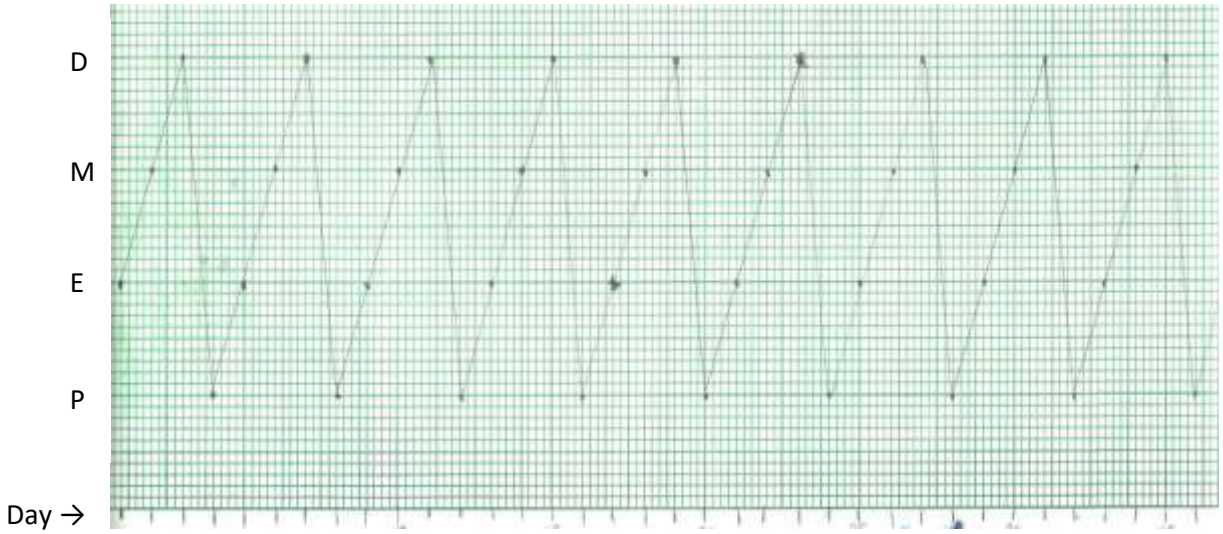


Fig. 4.1: Plots for daily smear obtained for Artemether over 39 days showing various stages of the oestrous cycle

Upper panel: Artemether (3.2 mg/kg loading dose & 1.6mg /kg maintenance dose), *i.p*. daily for 7 days.

Lower panel: 3% v/v Tween 80 in which artemether was suspended

P= proestrus, E= oestrus, M= metoestrus and D= dioestrus, adm=administration

Admin =administration

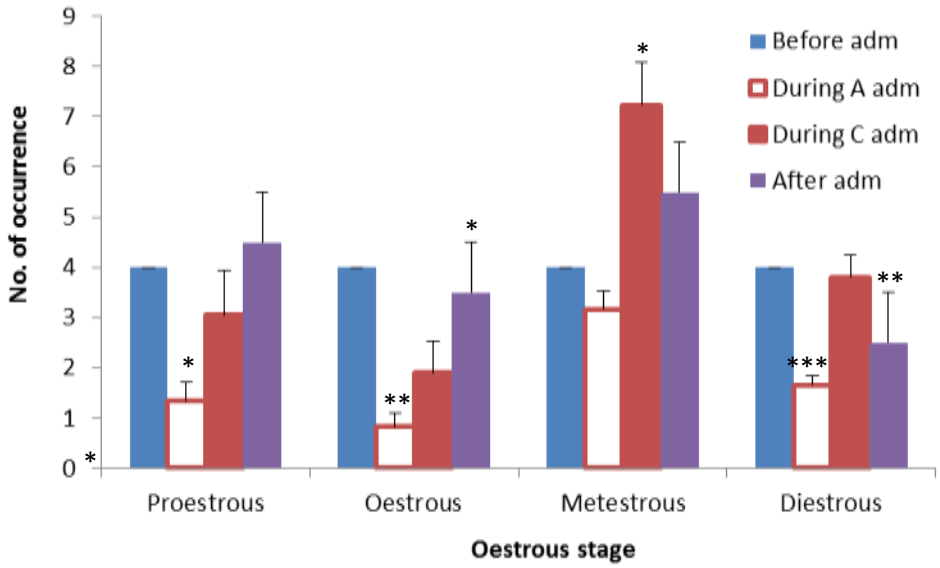


Figure 4.2: Occurrence of oestrous cycle stages in rats before, during and after artemether administration

During A: Artemether (32 mg/kg loading dose & 16 mg /kg maintenance dose) *i.p.* daily for 7 days
During C: corrected for 16- days utilized for before and after artemether administration
n=6 rats, Bar = mean ± SEM, *=P<0.05, ** = P<0.01, ***= P<0.001 (ANOVA, LSD post hoc), adm= administration

4.1.2 Chloroquine

Results obtained before, during and after administration of chloroquine (2.5 mg/kg [max.25 mg/ kg) are presented in appendix IV, figure 4.3, figure 4.4 and appendix V. There was desynchronization of the oestrous cycle but not as much as with artemether. Cycling has apparently returned to about normal by day 34 when the study ended. The metoestrus and proestrus stage were increased while the oestrus stage is decreased ($p<0.05$).

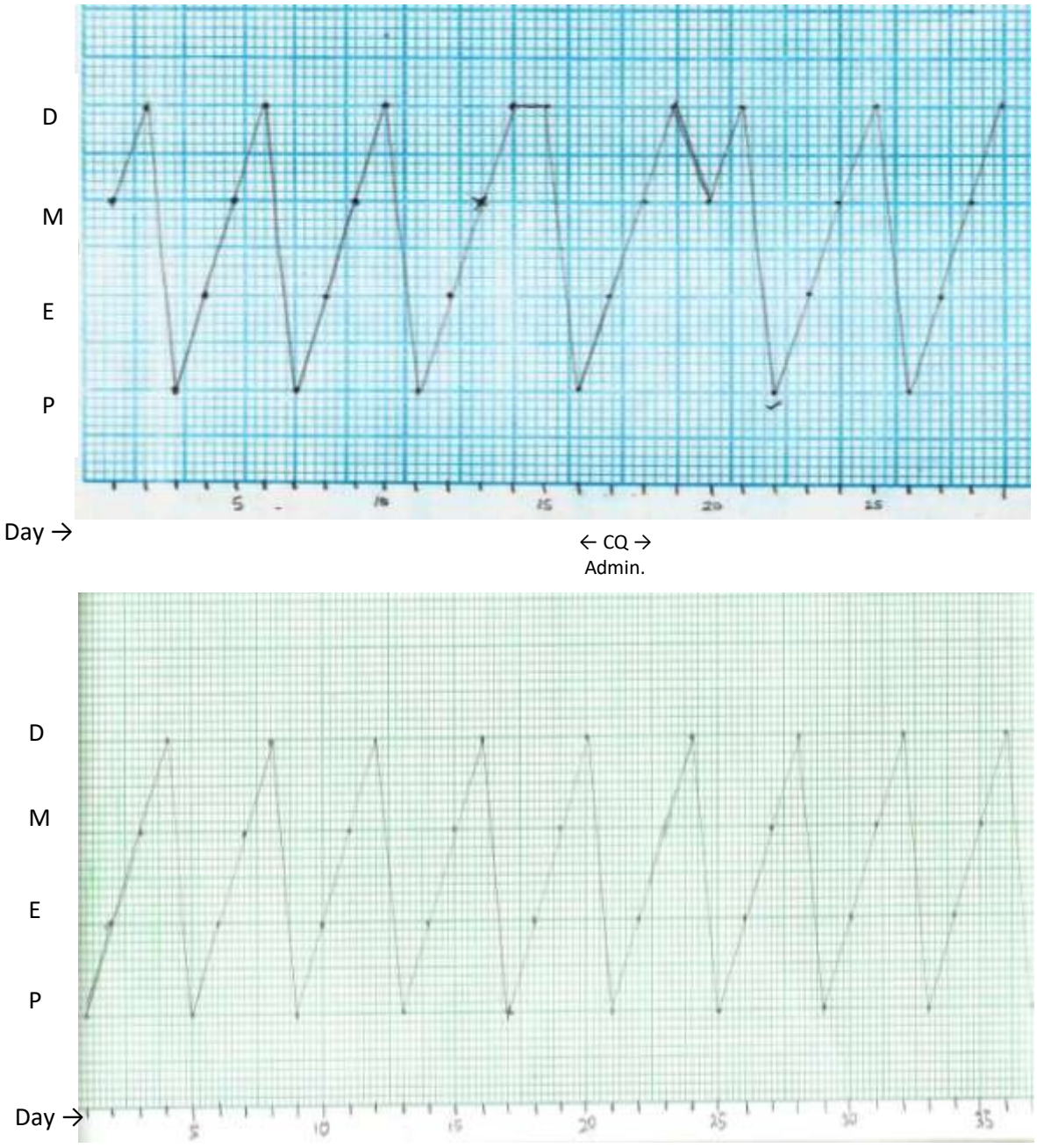


Fig. 4.3: Plot for daily smear obtained for Chloroquine over 34 days showing various stages of the oestrous cycle

Upper panel: Chloroquine (CQ) 2.5 mg/kg [max.25 mg/ kg] dose)
Lower panel: Distilled water in which chloroquine was dissolved
P= proestrus, E= oestrus, M= metoestrus and D= dioestrus CQ = Chloroquine
Admin = administration

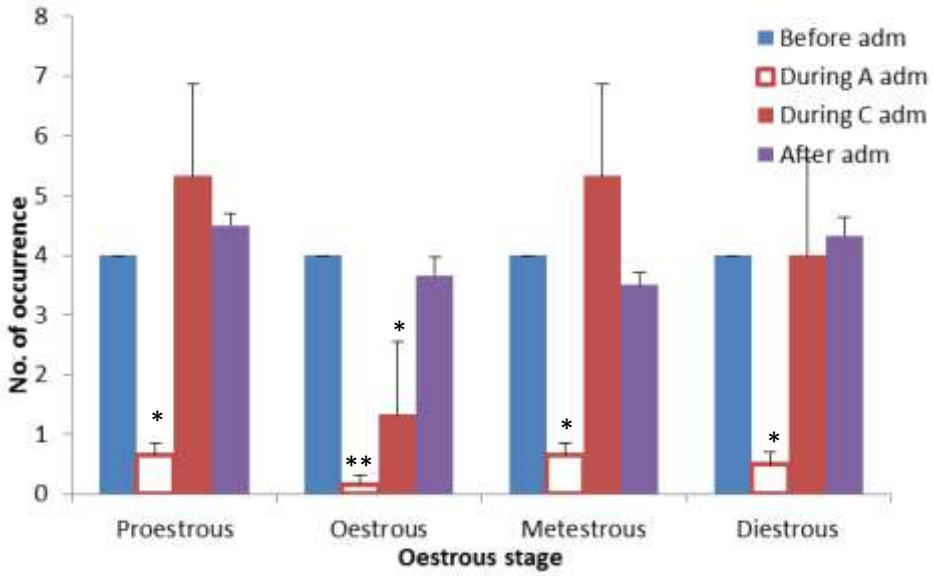


Figure 4.4: Occurrence of oestrous cycle stages in rats before, during and after chloroquine administration.

During A: (2.5 mg/kg [max.25 mg/ kg] dose), *i.p*
During C: corrected for 16- days utilized for before and after chloroquine administration
n=6 rats, Bar = mean ± SEM, *=P<0.05, ** = P<0.01, ***= P<0.001 (ANOVA, LSD post-hoc), adm= administration

4.1.3 Quinine

With regards to quinine administration (20 mg/kg loading dose & 10 mg /kg maintenance dose), there was desynchronization; the oestrous stage was particularly reduced ((p<0.001) while the proestrous and metoestrous had increased (p<0.01). Normal cycling had returned by day 30 (Appendix VI, figure 4.5, figure 4.6 and appendix VII).

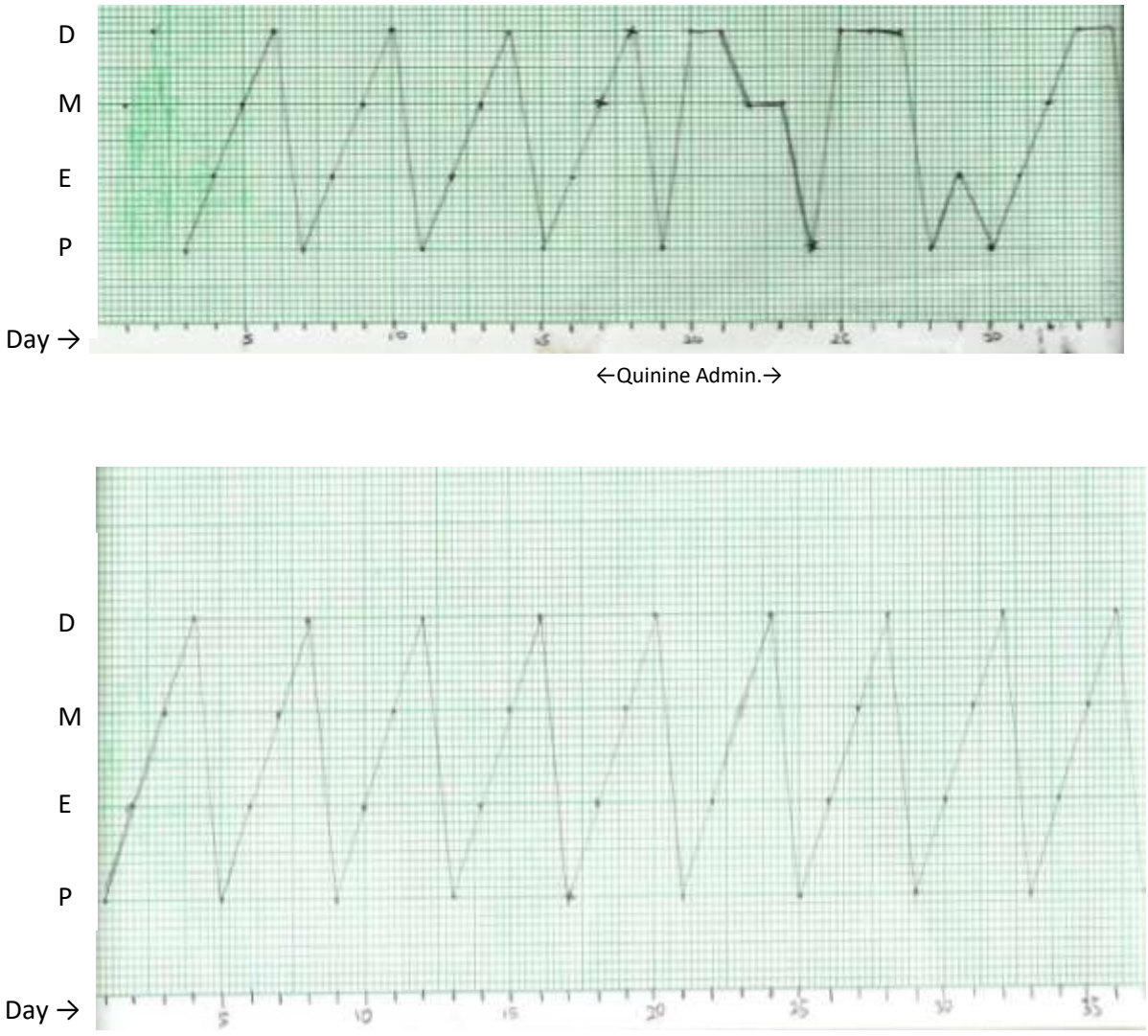


Fig. 4.5 Plot for daily smear obtained for Quinine over 40 days showing various stages of the oestrous cycle

P = proestrus, E = oestrus, M = metoestrus and D = dioestrus
Upper panel: Quinine (20 mg/kg loading dose & 10 mg /kg maintenance dose), i.p. daily for 7 days
Lower panel: Distilled water in which Quinine was dissolved
Admin =administration

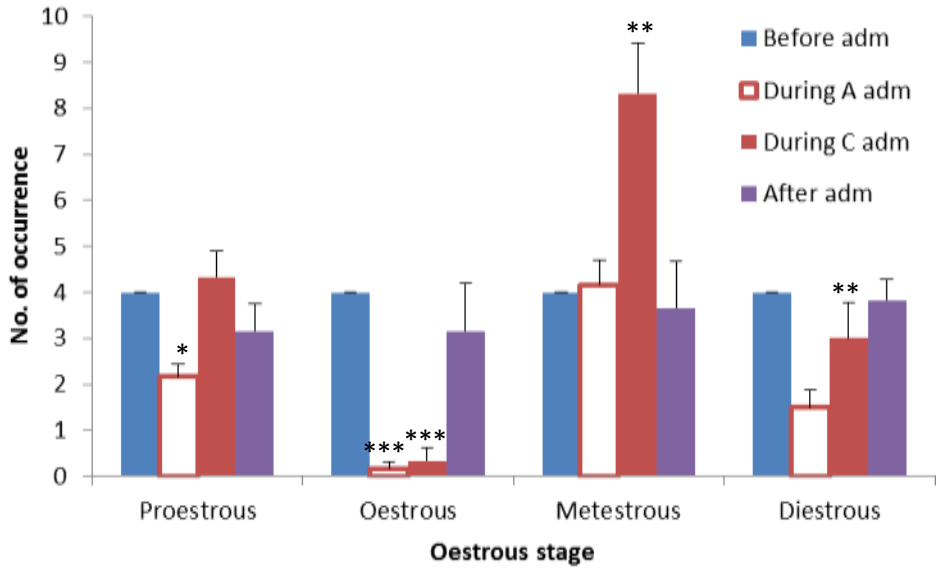


Figure 4.6: Occurrence of oestrous cycle stages in rats before, during and after quinine administration

During A: Quinine (20 mg/kg loading dose & 10 mg /kg 8 hourly) *i.p*. daily for 7 days
During C: corrected for 16- days utilized for before and after quinine administration
n=6 rats, Bar = mean ± SEM, *=P<0.05, ** =P<0.01, ***= P<0.001 (ANOVA, LSD post hoc), adm=administration

4.1.4 sulphadoxine/pyrimethamine

Result obtained for treatment with sulphadoxine/ pyrimethamine (25 mg/kg/1.25 mg/ kg) showed desynchronization from the normal oestrous cycle (fig. 4.7). All the stages were decreased except proestrus which was increased but the (appendix VIII, figures 4.7 and 4.8 and appendix IX).

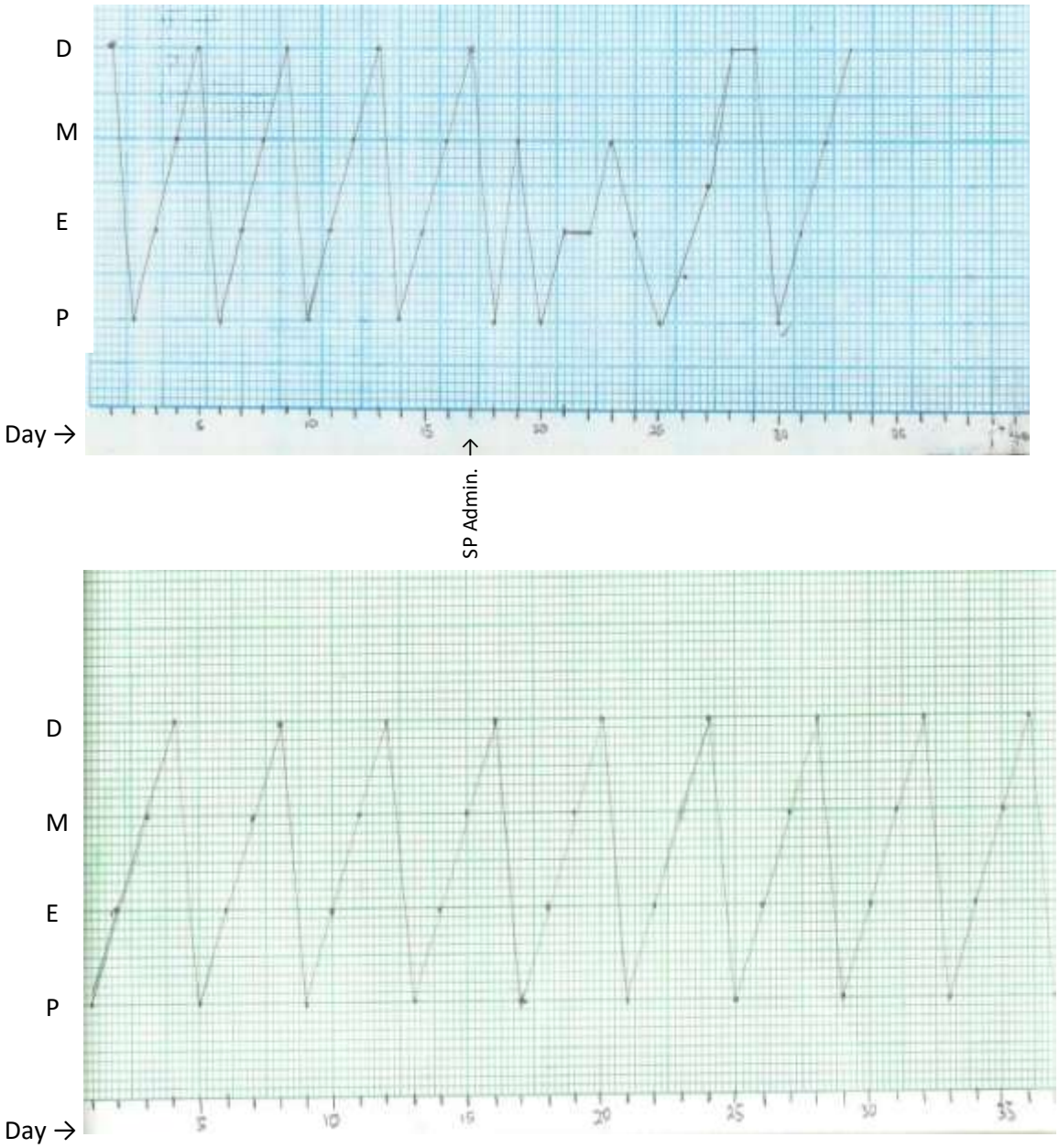


Fig. 4.7 Plot for daily smear obtained for Sulphadoxine-Pyrimethamine over 33 days showing various stages of the oestrous cycle

P= proestrus, E= oestrus, M= metoestrus and D= dioestrus
Upper panel: Sulphadoxine-Pyrimethamine (SP)(25 mg/kg/1.25 mg/kg), *i.p*. once
Lower panel: Distilled water in which Sulphadoxine-Pyrimethamine was dissolved
Admin =administration

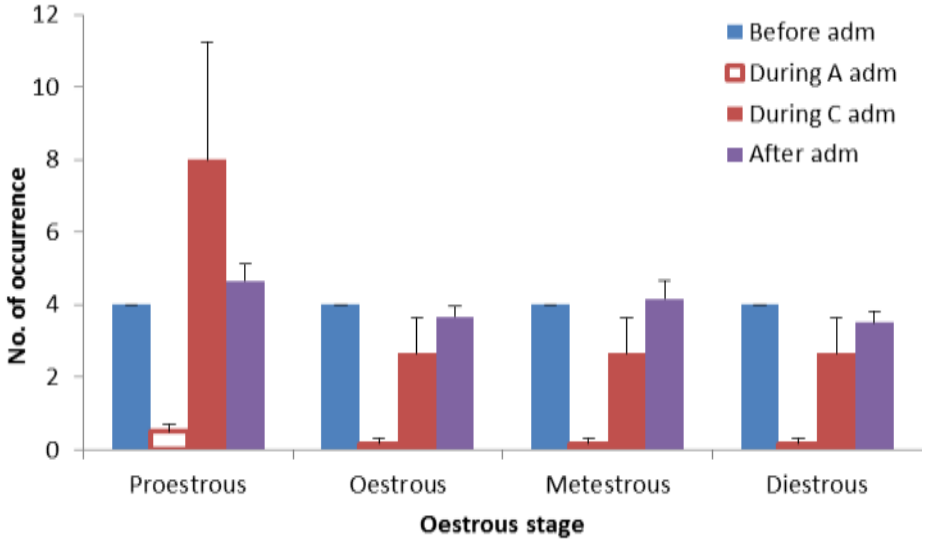


Figure 4.8: Occurrence of oestrous cycle stages in rats before, during and after Sulphadoxine/Pyrimethamine administration

During A: Sulphadoxine/Pyrimethamine (25 mg/kg/1.25 mg/kg) *i.p.* once
During C: corrected for 16- days utilized for before and after SP
n=6 rats, Bar = mean ± SEM, No statistical significance, adm= administration

4.1.5 Comparison of the effects of the antimalarial on the stages of the oestrous cycle

Comparing the effect of all the antimalarial drugs on the various stages of the oestrous cycle, reveals that the proestrus stage was generally increased especially by SP (but decreased for AT) when the antimalarials were administered; on the other hand, the oestrus stage was reduced by the antimalarials in the order of QN >CQ > AT > SP (figure 4.10 and Appendix XI). The metoestrus was increased by all the antimalarials except SP which was decreased although return to normal was apparent at the end of the study (figure 4.11 and Appendix XII). Administration of CQ did not significantly affect the dioestrus but with AT, QN and SP, there was decrease dioestrus with normalization at the end of the study approximating for all but AT (figure 4.12 and Appendix XII). Thus, CQ seemed to have affected the oestrus cycle the least while SP affected it the most, with AT and QN in between. (figures. 4.9- 4.12 and appendices X-XIII)

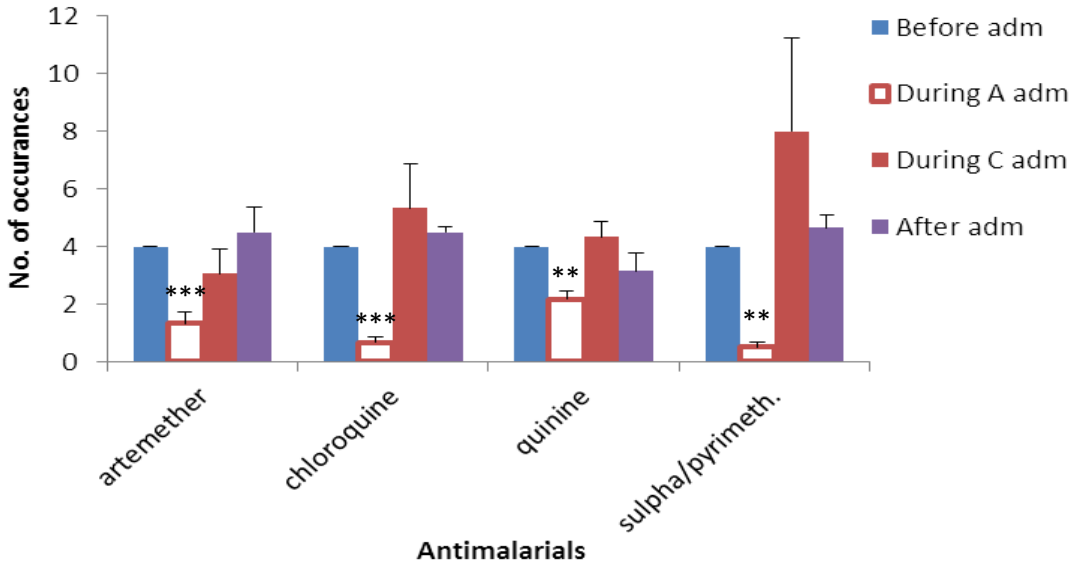


Figure 4.9 Proestrus stage after administration of antimalarials

Actual data refers to the period of antimalarial administration
Corrected for 16- days utilized for before and after antimalarial administration
n=6 rats, Bar =mean ± SEM, ** = P<0.01, ***= P<0.001 (ANOVA, LSD post hoc), adm=administration

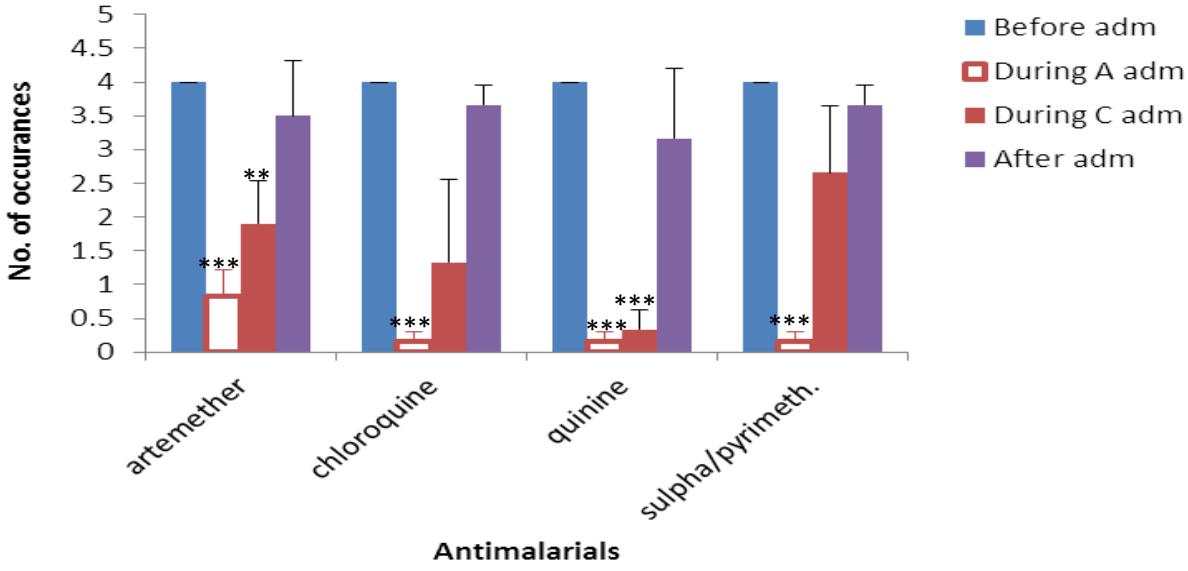


Figure 4.10 Oestrus stages after administration of antimalarials

Actual data refers to the period of antimalarial administration
Corrected for 16- days utilized for before and after antimalarial administration
n=6 rats, Bar =mean ± SEM, ** = P<0.01, ***= P<0.001 (ANOVA, LSD post hoc), adm=administration

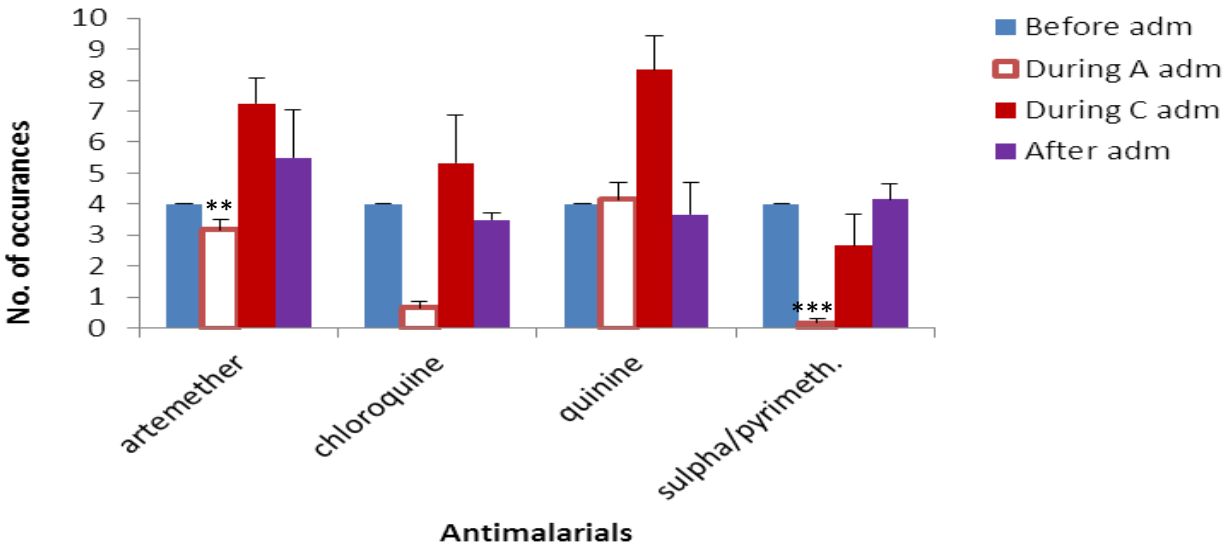


Figure 4.11 Metoestrus stages after administration of antimalarials

Actual data refers to the period of antimalarial administration
Corrected for 16- days utilized for before and after antimalarial administration
n=6 rats, Bar =mean ± SEM, ** = P<0.01, ***= P<0.001 (ANOVA, LSD post hoc), adm=administration

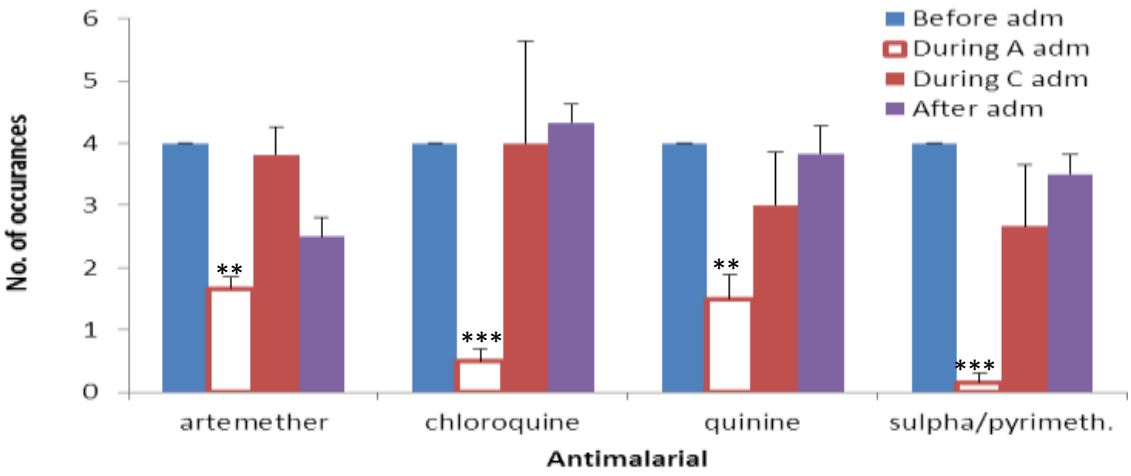


Figure 4.12 Dioestrus stages after administration of antimalarials

Actual data refers to the period of antimalarial administration
Corrected for 16- days utilized for before and after antimalarial administration
n=6 rats, Bar =mean ± SEM, ** = P<0.01, ***= P<0.001 (ANOVA, LSD post hoc), adm=administration

4.2 Effects of the Antimalarial Drugs and oxytocin on Contractility of the Pregnant Rat Uterus

4.2.1 Oxytocin

The effects of the antimalarial drugs as well as the reference drug oxytocin were as shown below. Oxytocin (10-80 ng/ml) concentration-dependently contracted the quiescent pregnant rat uterus affirming tissue viability (figure 4.13).

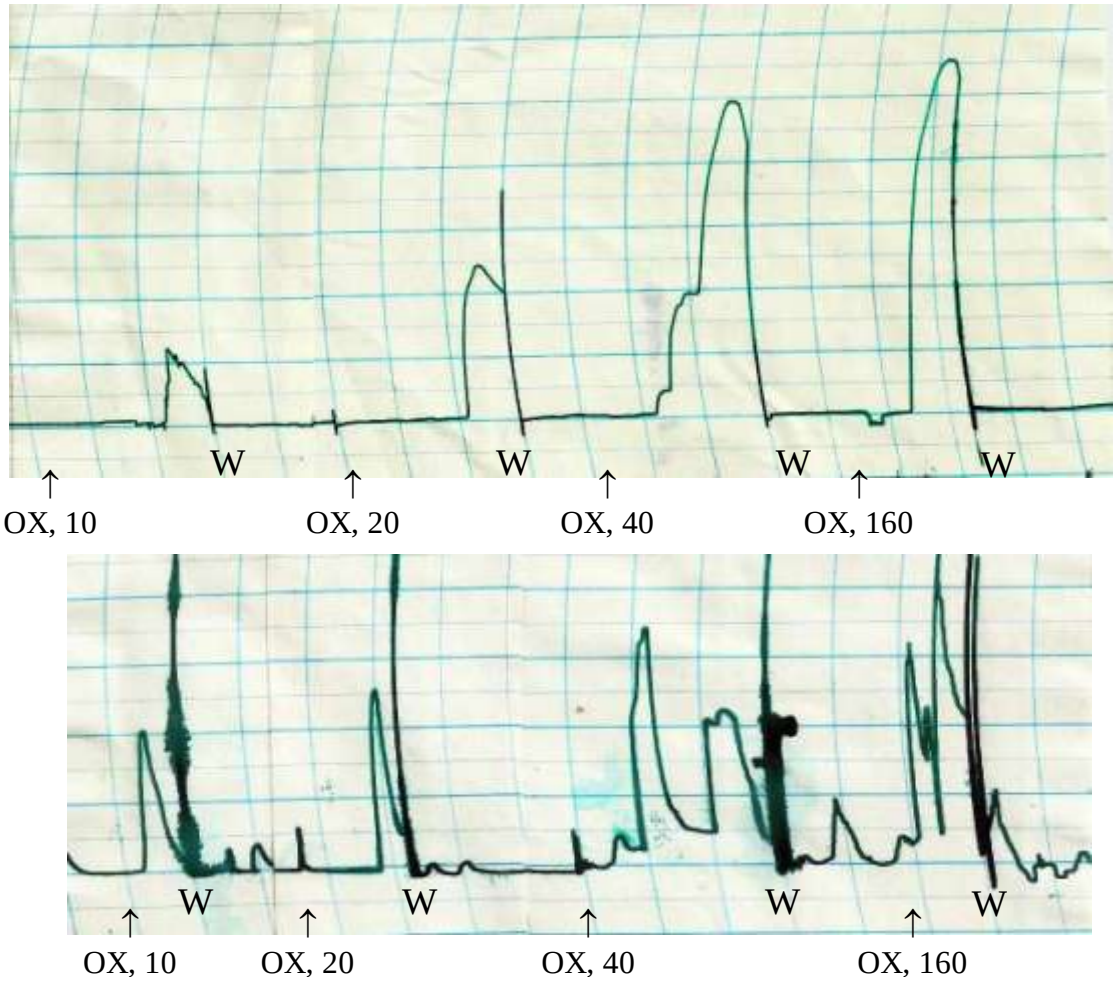


Figure 4.13: Contractile effect of Oxytocin in isolated pregnant rat uterus

Upper panel: Quiescent preparation;
Lower panel: Non-quiescent preparation
Physiological Salt Solution: De Jalon
OX= Oxytocin, organ bath concn. in ηg/ml; W= Washing

4.2.2 Artemether

At concentration of 16-1280 µg/ml, artemether did not produce any effect on the isolated pregnant uterus figure 4.14

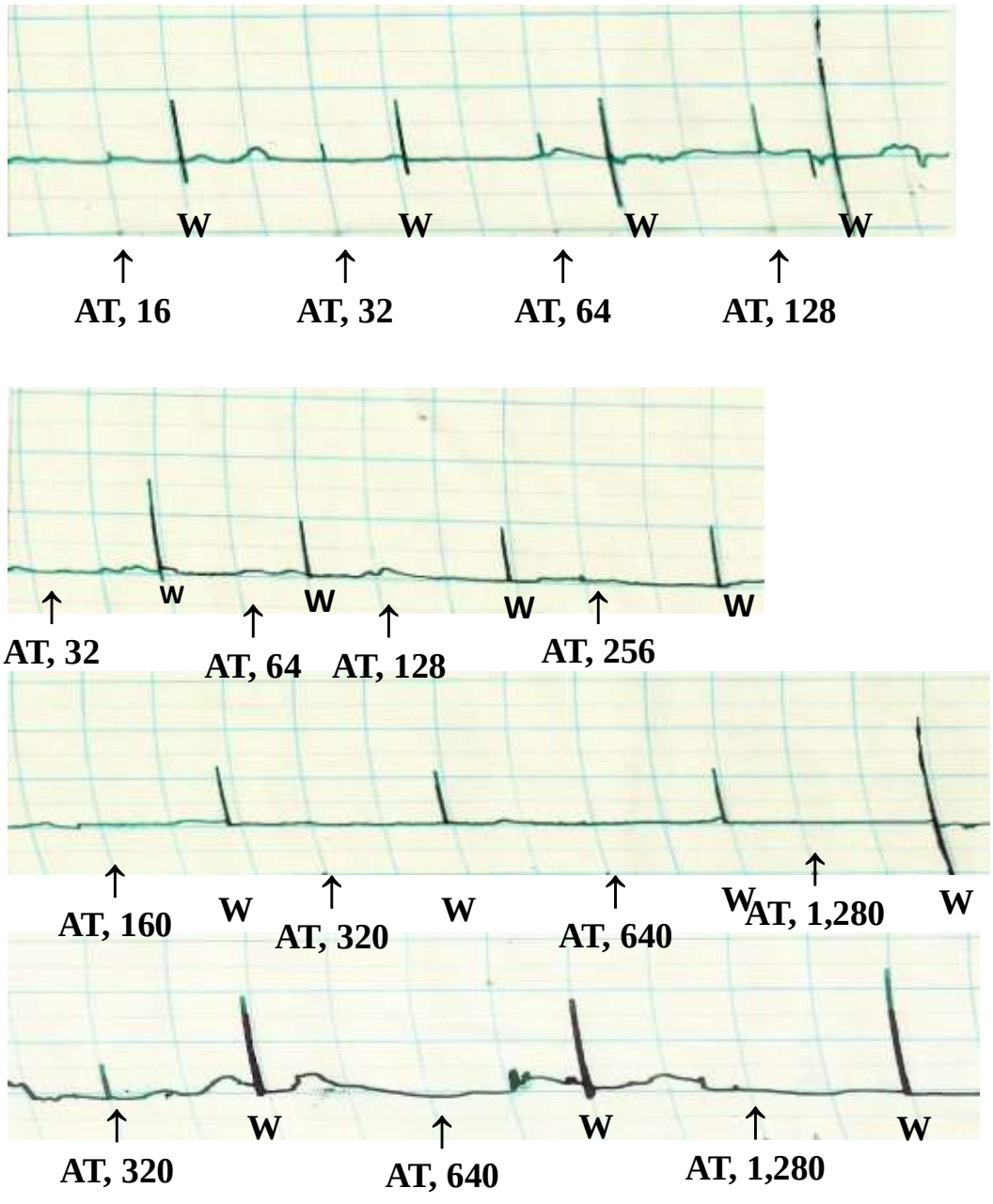


Figure 4.14: Effect of Artemether on the isolated pregnant rat uterus

Physiological Salt Solution: De Jalon
AT= Artemether, organ bath concn. In µg/ml; W= Washing

However, when combined with oxytocin, artemether attenuated contractile effect of oxytocin (80-160 ng/ml) figure 4.15

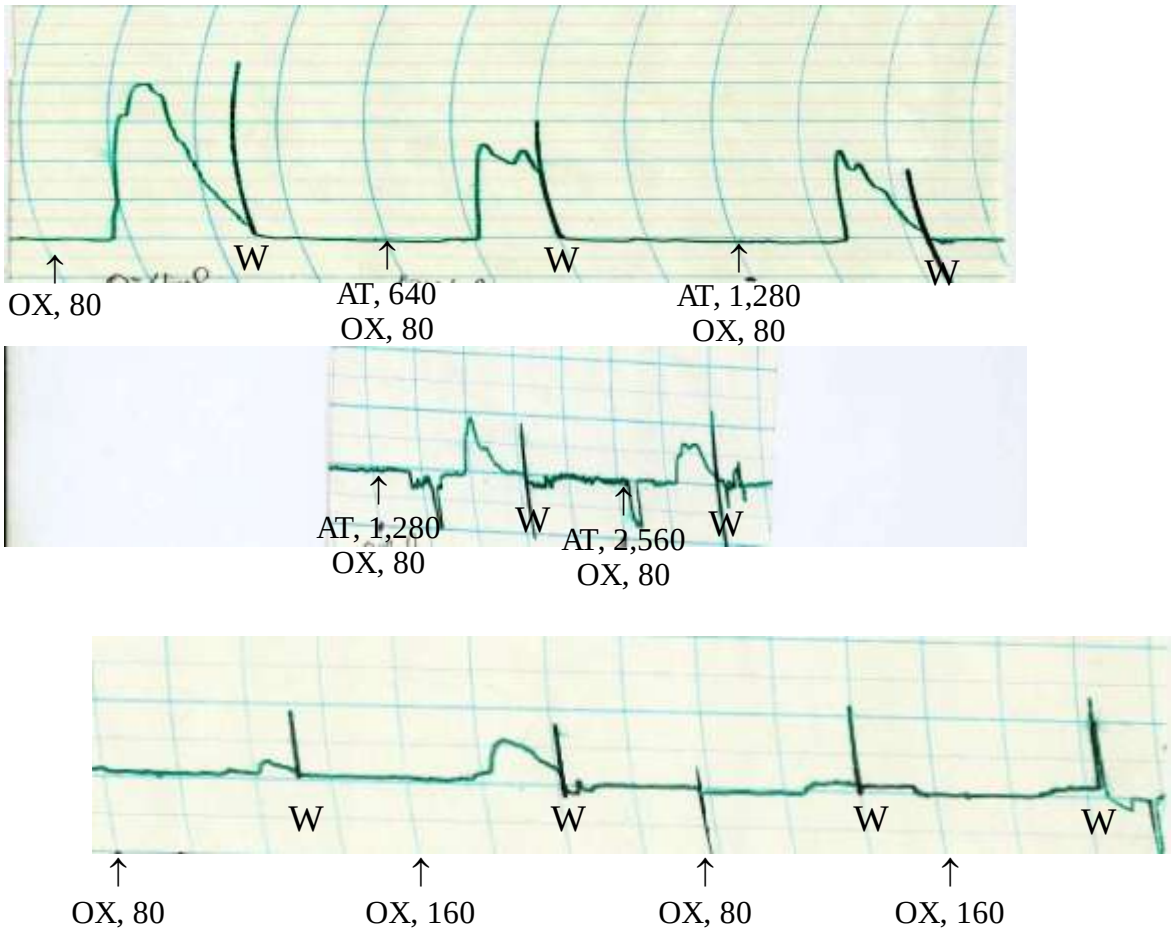


Figure 4.15: Effect of artemether and oxytocin on the isolated pregnant rat uterus

Panels are continuous; Physiological Salt Solution: De Jalon; W= Washing
AT= Artemether, organ bath concn. In μg/ml; OX= Oxytocin, ηg/ml
Lower panel is continuation of upper panel

4.2.3 Chloroquine

Similarly, chloroquine (4-8 mg/ml) did not have contractile effect on isoloated pregnant uterus but appears to diminish oxytocin induced contraction of the pregnant rat uterus (figure 4.16 and 4.17)

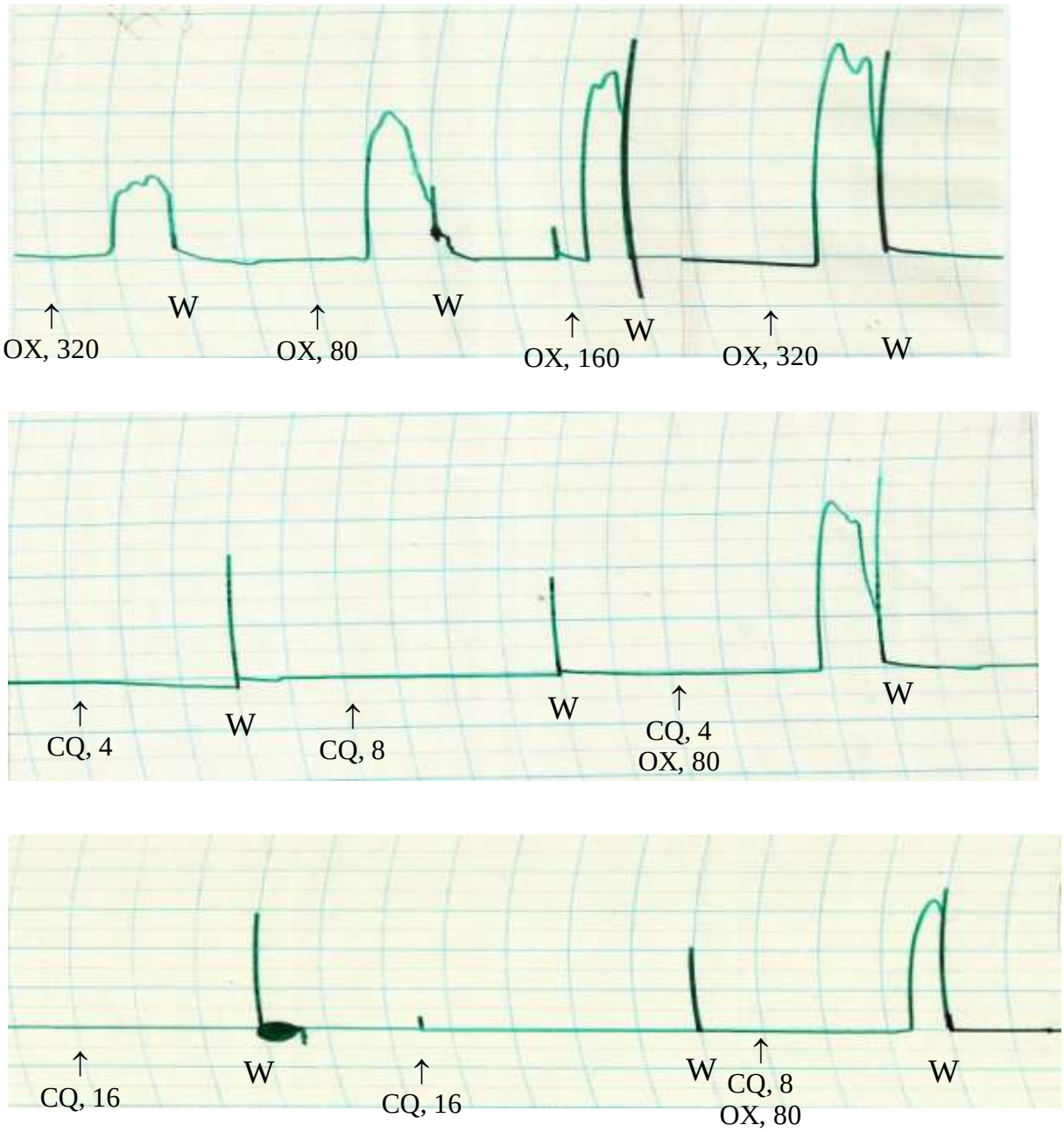


Figure 4.16: Effect of chloroquine on the isolated pregnant rat uterus

Physiological Salt Solution: De Jalon; W= Washing
CQ= Chloroquine, organ bath concentration in mg/ml; OX= Oxytocin, ηg/ml

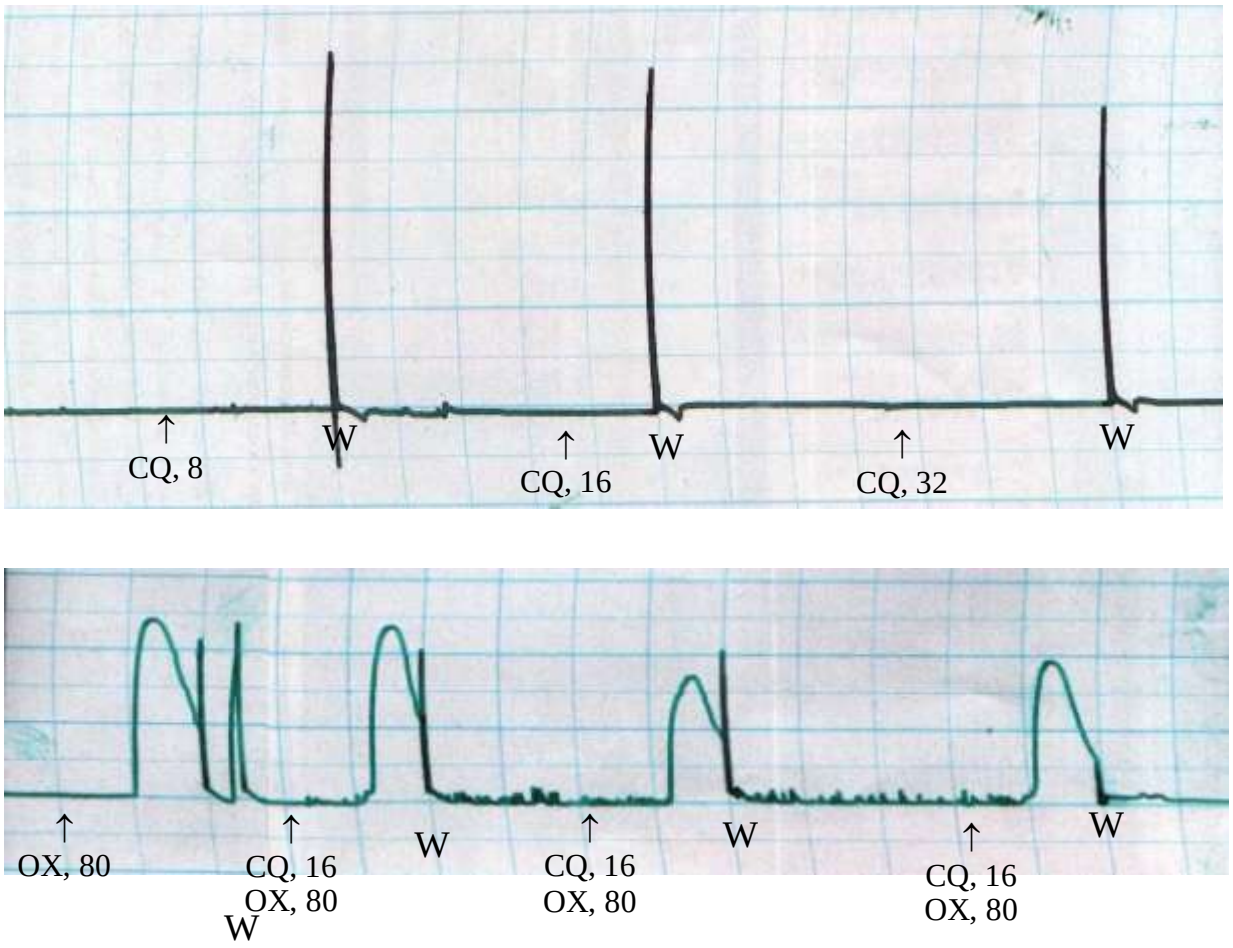


Figure4. 17: Effect of chloroquine and oxytocin reducing the effect of Oxytocin on the isolated pregnant rat uterus

Panels are continuous; Physiological Salt Solution: De Jalon; W= Washing
CQ= Chloroquine, organ bath concentration in mg/ml; OX= Oxytocin, ηg/ml

4.2.4 Quinine

Quinine did not show contractile or relaxant effect on the isolated pregnant rat uterus (Figure 4.18).

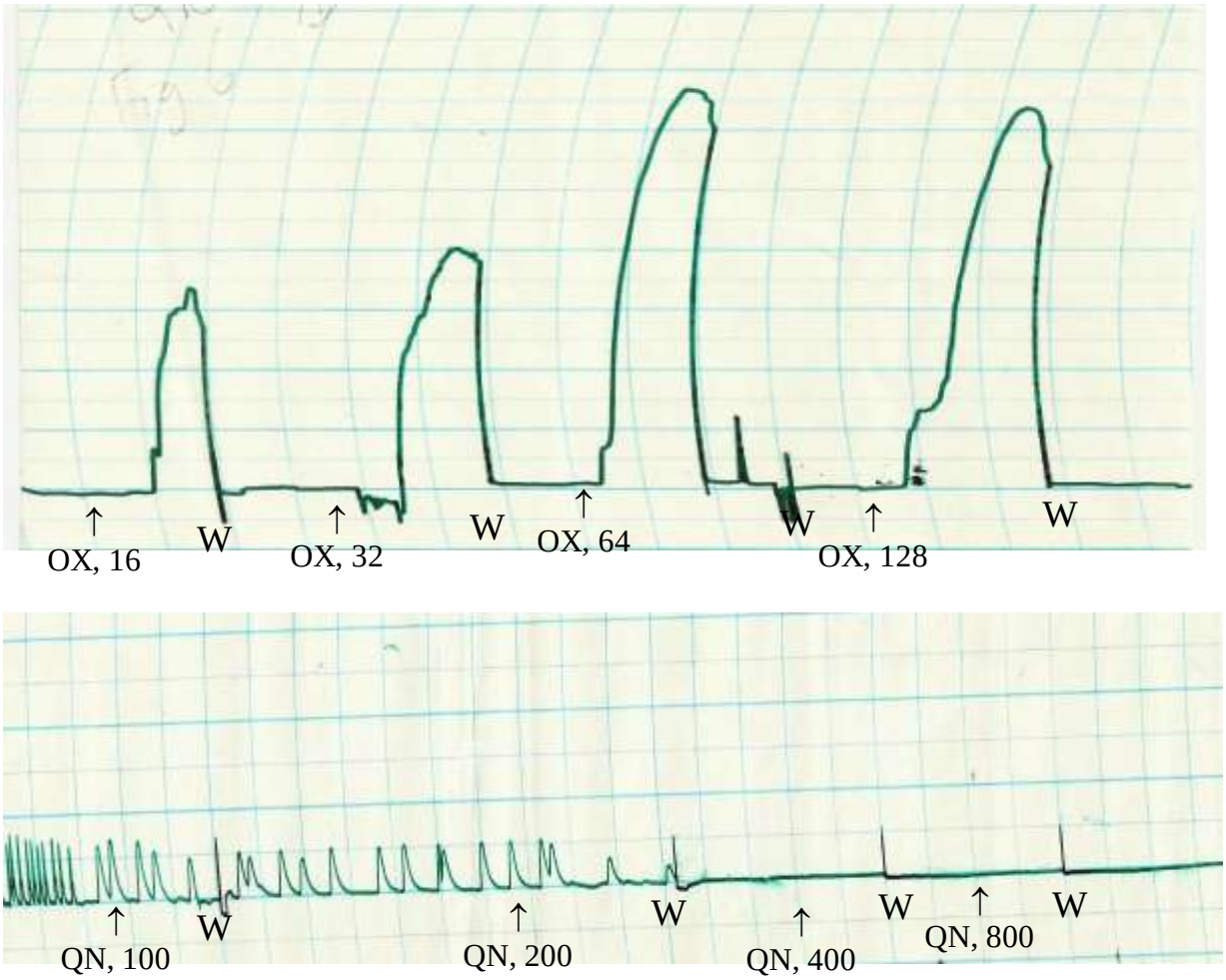


Figure 4.18: Effect of quinine isolated pregnant rat uterus

Panels are separate preparations; Physiological Salt Solution: De Jalon; W= Washing
QN= Quinine, organ bath concentration in μg/ml; OX= Oxytocin, ηg/ml

4.2.5 Sulphadoxine/ pyrimethamine

The effect produced by SP is similar to that of artemether and chloroquine they did not produce contractile effect on pregnant rat uterus but was shown to attenuate oxytocin induced contractile of the organ (Figure 4.19).

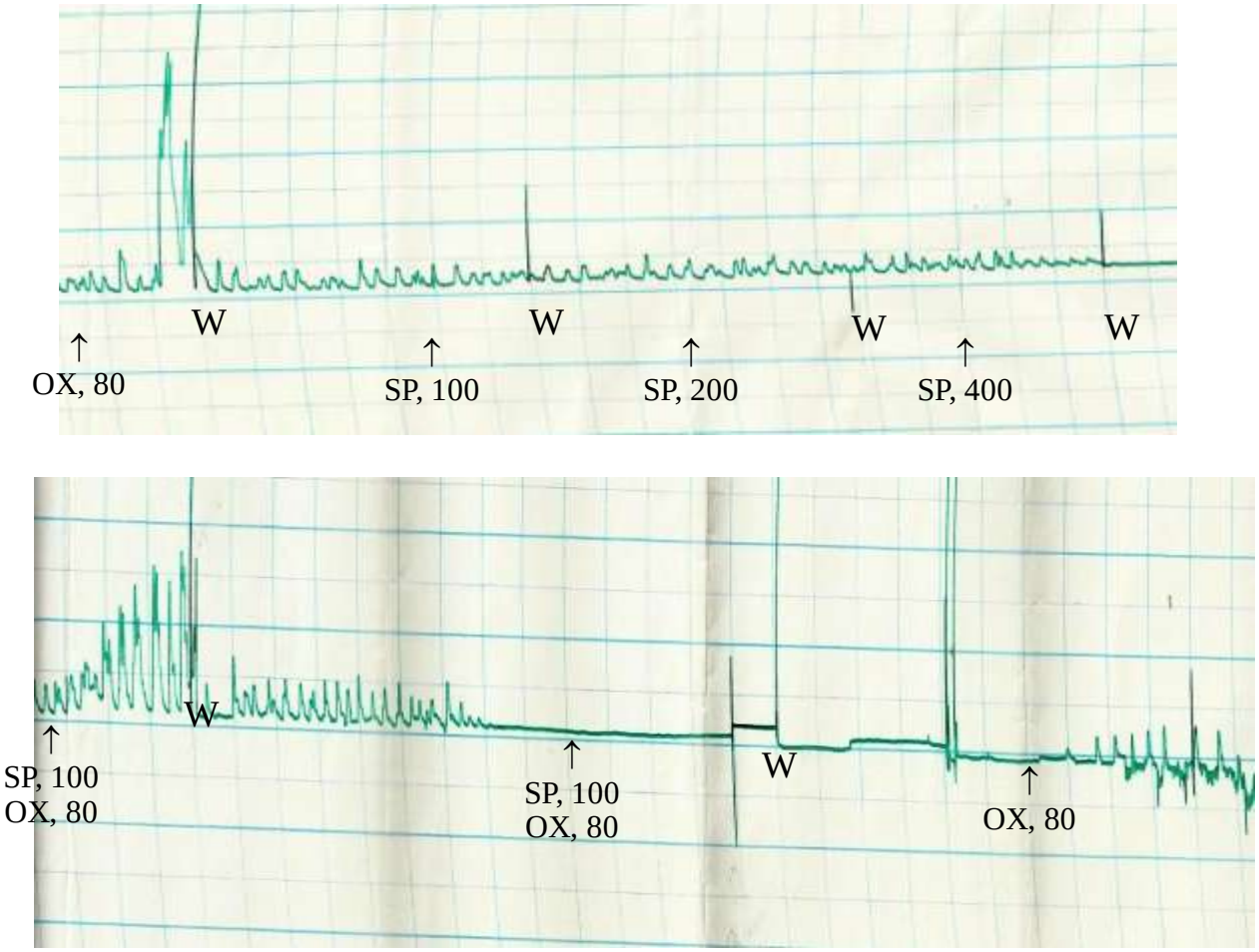


Figure 4.19: Effect of Sulphadoxine-Pyrimethamine isolated pregnant rat uterus

Panels are separate preparations; Physiological Salt Solution: De Jalon; W= Washing
SP= Sulphadoxine-Pyrimethamine, organ bath concentration in mg/ml; OX= Oxytocin, ηg/ml
Upper panel: SP having no effect on the isolated pregnant rat uterus
Lower panel: SP and OX diminishing the contractile effects of Oxytocin

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of Antimalarials on the Oestrous Cycle in Wistar Rats

The wistar rats used in this study were cycling normally (4 days) before treatment with the antimalarial drugs. But during and after treatment, desynchronization from the normal cycling pattern was observed for all the antimalarial drugs. Each phase of the oestrus cycle has its special role in reproduction. The proestrus is the phase of follicular growth and maturation under the influence of the increasing level of estrogen, while the oestrus phase, which is known as the heat period, is characterized by ovulation which occurs under the influence of high level of estrogen which occurs as a result of LH surge. The metoestrus and dioestrus phase, are notable for increasing level of progesterone by corpus luteum which is required for the preparation of the endometrium for possible implantation of fertilized ovum and subsequently for maintenance of pregnancy if fertilization occurs. In the absence of pregnancy, the corpus luteum regresses a drop in level of progesterone,, this drop causes a positive feedback on the hypothalamus leading to increase release of FSH and LH by the pituitary gland which in turn brings about the build-up in the concentration of estrogen as well as progesterone by the ovaries and the next cycle begins.

In the present study following the administration of artemether, desynchronization was observed with the proestrus phase decreased. The proestrus stage is where growth and maturation of the follicle occur under the influence of increasing levels of oestrogen, the decrease in proestrus phase observed in this study would mean a decrease in the level of estrogen which in turn would affect the process of growth and development of the follicle. A number of explanations have been offered to explain

the reason for decrease in proestrus stage being due to low level of gonadotropin (Goldman *et al.,* 1997; Abiodun, *et al.,* 2012, Oyedeji, *et al.,* 2013). The possible implication of this finding is fertility compromise, as low level of gonadotropin will affect the growth and maturation of the follicle.

This finding is at variance with that reported by Ejiofor *et al.,* (2006) in which artemether did not affect the normal cycling pattern during and after administration of the drug. This might possibly be due to the doses of artemether used in the studies of which, the highest dose administered daily (15mg/kg) was less than the daily maintenance dose (16mg/kg) used in this present study.

Upon administration of chloroquine, desynchronization was also observed. The oestrus phase was decreased implying a low level of oestrogen. Therefore, the process of ovulation which requires a high level of oestrogen to occur will be hindered. Though there is increase in both proestrus and metoestrus phase. The metoestrus phase is where progesterone level starts building up, preparing the endometrial lining for possible implantation. But, if the ovulatory process is hindered, the process of fertilization and subsequent implantation will also be hindered and in totality fertility affected. But it is worthy to mention that by the end of the study period, normal cycling resumed implying that, the observed effect was temporary.

Administration of quinine on the other hand also showed a desynchronization from the normal pattern with oestrus phase decrease at $P<0.001$. Just as with chloroquine the process of ovulation will also be affected or an anovulation cycle might be obtained as a consequence of quinine administration.

Sulphadoxine/pyrimethamine also produces desynchronization of the normal oestrous cycle causing a decrease of all the phases except the proestrus stage which was

increased. This might mean an increase or build-up of oestrogen level but the decrease observed for the other stages would cause decrease in the level of gonadotropin associated with those phases thereby creating an imbalance in the level of gonadotropin. This implies that Sulphadoxine/pyrimethamine might have an effect on fertility.

5.2 Effect of Antimalarials on Uterine Contractility of Wistar Rats

Oxytocin is used in the induction or Augmentation of labour as it acts directly on uterine smooth muscles to increase uterine contractions and it also stimulates the formation of prostaglandins, which also enhances the oxytocin–induced contractions (Phaneuf *et al.,* 2000). Contraction elicited by oxytocin in a concentration dependent manner ascertains the tissue's viability.

Artemether (16mg – 1280mg/ml) did not produce any effect when used alone on the isolated pregnant uteri of Wistar rat, suggesting that artemether is not likely to affect uterine activity in the non-pregnant state (fig 4.14). However, artemether (640-2560ng/ml) was found to reduce oxytocin-induce contraction of the isolated pregnant rat uteri. The implication of this finding is that, since rats are frequently used in reproductive studies that are extrapolated to human, this effect of artemether suggests that its use in late pregnancy or during parturition may inhibit the initiation and progress of labour and also the outcome of labour. Prolonged labour causes undesirable consequences such as uterine rupture, delayed placenta expression, postpartum haemorrhage and failure of uterine contraction (Kaye *et al.,* 2014). This finding is the same with that of Ejiofor *et al.,* 2006 on the effect of artemether on the uterine smooth muscle of both pregnant and no pregnant rats.

Similarly, chloroquine (4-16mg/ml) did not produce any effect on the isolated pregnant uteri when used alone but at organ bath concentration of 4-8mg/ml it was found to reduce oxytocin–induced contractions suggesting an untoward effect when used during late pregnancy or during labour and also affect the outcome of pregnancy.

This is at variance with the findings of Nwaigwe *et al.,* (1997) where chloroquine was found to completely abolish oxytocin-induced contraction. This might be as a result of the dose of chloroquine (12.7ng/ml) used for that study which was higher than in the present study (4-8ng/ml).

Quinine (100-800ng/ml) did not produce any effect on the pregnant uteri of rats suggesting no likely effect when used pregnant and non-pregnant state. Incidentally, studies with quinine suggest that abortifacient activity attributed to quinine is due to toxic doses used and not due to increased uterine activity (Dannenberg *et al.*, 1983).

Sulphadoxine/pyrimethamine (100 – 400ng/ml) also produced no effect when used alone on the pregnant uteri but was able (at 100ng/ml) to reduce oxytocin – induced contraction. This could translate to possible ill effect on initiation and maintenance of labour and as well as outcome of labour.

Although, these antimalarials are used for the treatment or prophylaxis of malaria, it should be borne in mind that they may interfere with menstrual cycle when used by women in their reproductive age and also interface with initiation and maintenance of normal contractile rhythm of the uterus when used at ate pregnancy and during labour.

CHAPTER SIX

6.0 Conclusion and Recommendation

6.1 Conclusion

In this study, the antimalarial drugs caused a desynchronization of the normal cycle especially at the proestrous and oestrous which are generally decreased, metoestrous stage was increased and dioestrous was least affected. QN appeared to have resulted in the most effect while SP was the least. Several studies on traditional plants as well as orthodox medicines (antimalarials) have been shown to decrease gonadotropin levels causing similar effects on the oestrous cycle stages as observed in this present study (Ksheerasagar *et al.,* 2008, Agwai and ugwu, 2012, Abiodun *et al.*, 2013).

This suggests that these antimalarial can be administered to women of child bearing age but bearing in mind the possible interference with the menstrual cycle as they might affect the oestrogen progesterone balance.

No agonist effect was evident for all the antimalarial drugs but a reduction in oxytocin induced contractility was observed. Therefore, the use of those drugs during pregnancy might affect the onset, progress and subsequent outcome of labour (e.g. ruptured uterine) when used at late stage of pregnancy progress of labour or during labour though, there are no effect on the uterus when those drugs were used alone.

6.2 Recommendation

Relating to the findings from this study, we recommend that more research be carried out to confirm the effects of these antimalarial drugs on hormonal levels in laboratory animals possibly primates. The effects of these drugs should be studied on the various trimesters of pregnancy to evaluate their effect on the pregnant uteri.

Finally, further studies of the effects of these antimalarial drugs on malaria infected animal should also be studied.

REFERENCE

Abiodun, A.A., Falana, B.A. and Oyeyipo, I.P. (2012). Changes in oestrous cycle of adult female wistar rats treated with artemether. *Journal of Neuroscience and Behavioural Health*; 4(3): 15-19.

Agbai, E.O. and Ugwu, N.F. (2012). Dose Department effect of aqueous *cola nitida rubia* extracts on reproductive functions in female albino Wister rats in Elele. *Journal of Biological Sciences and Bioconservation;* 4:82-92.

Akintowa, A., Meyer, M.C. and Yau, M.K. (1983). Placental transfer of chloroquine in pregnant rabbits. *Research Communication in Chemical Pathology and Pharmacology*; 40(3): 443-455.

Allen, E. and Doisy, E.A. (1923). An Ovarian hormone. Preliminary reports on its localization, extraction and partial purification and action in test animals. *Journal of American Medical Association;* 81:819-821.

Allen, F. (1922). The oestrous cycle in the mouse. *American Journal of Anatomy* 30: 297-371.

Allen, S.J., Raiko, A., O'donnell, A., Alexander, N.D. and Clegg, J.B. (1998).Causes of preterm delivery and intrauterine growth retardation in a malaria endemic region of Papua New Guinea. *Archive of Diseases and Child Fetal and Neonatal Edition;* 79(2*):* 135-140.

Archibald, H.M. (1956). The influence of malaria infection of the placenta in the incidence of prematurity. *Bulletin of WHO*; 15:842-845.

Astwood, E.B. (1939). Changes in weight and water content of uterus of the normal adult rat. *American Journal of Physiology*.126: 162-170.

Balaka, B., Aghere, A.D., Bonkoungou, P., Kessie, K., Assimadi, K. and Agbo, K. (2000). Congenital malarial disease due to *Plasmodium falciparum* in high infection risk newborn. *Archives of Paediatrics*; 7(3); 243-248.

Barradell, L.B. and Fitton, A. (1995). Artesunate: A review of its pharmacology and therapeutic efficacy in the treatment of malaria. *Drugs*; 50(4): 714-41.

Beyens, M.N., Guy, C. Ratrema, M. and Ollagnier, M. (2003). Prescription of drugs to pregnant women in France: the HIMAGE Study. *Therapie* 58: 505-511.

Bloland, P. B., Kazembe, P. N., Oloo, A. J., Himonga, B., Barat, L. M., Ruebush, T. K. (1998). Chloroquine in Africa: critical assessment and recommendations

for monitoring, evaluating chloroquine therapy efficacy in sub- Saharan Africa. *Tropical Medicine and International Health;* 3 (7): 543-552.

Bouree, P. (1997). Malaria: what treatment today? *Presse Medicine*; 26(4): 156-157.

Bouree, P.,Taugourdeau, P.H. and Anh, Van N.G. (1994). *Malaria*. Smithkline Beecham pharmaceuticals,Middlesex, U.K.; 1-40.

Brabin, B.J. (1983). An analysis of malaria in pregnancy in Africa. *Bulletin of World Health Organisation;* 61: 1005-1016.

Brewer, T.G., Grate, S.J., Peggins, Y.O., Weins, P.J., Petras, J.M., Levine, B.S..Heiffer, M.H. and Schuster, B.G. (1994). Fatal neurotoxicity of arteether and artemeter. *American Journal of Tropical Medicine and Hygiene*; 51: 251-259.

Bricaire, F., Salmon, D., Danis, M. and Gentilini, M. (1991). Antimalarials and Pregnancy. *Bulletin of the society of pathologists exotic;* 84(5): 721-738.

Bruce-Chwatt, L.J. (1951). Malaria in Nigeria. *Bulletin of WHO* 4: 301-327.

Bunnag, D., Kanda, T., Karbwang, J., Thimasarn, K., Pungpak, S. and Harinasuta, T. (1996). Artemether or artesunate followed by mefloquine as a possible treatment for multidrug resistant *falciparum* malaria. *South East Asian Journal of Tropical Medicine and Public Health*; 24(1): 49-52.

Bunnag, D., Karbwang, J., Chitames, S. and Harmnasuta, T. (1993). Intramuscular artemether in female patients with uncomplicated *faciparum* malaria. *South East Asian Journal of Tropical Medicine and Public Health;* 24(I): 49-52.

Butcher, R.L., Collin, W.E., and Fugo, N.W. (1974). Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day oestrous cycle of rat. *Endocrinology*; 94 (6): 1704-1708.

Byers, S.L., Wiles, M.V., Dunn, S.L. and Taft, R.A.(2012). Mouse estrous cycle identification tool and images. doi: 10.1372/ journal.pone. 0035538. g005

Caligioni, C.S. (2009). Assessing reproductive status/ stage in mice. *Current protocols in neuroscience*. doi:10.1002/0471142301.nsa04is48

Carswell, H.V., Anderson, N.H., Clark, J.S., Graham,D., Jeffs, B., Dominiczak, A.F., Macrae, I.M.(1999). Genetic and gender influences on sensitivity to focal cerebral ischemia in the stroke-prone spontaneously hypertensive rat; *Hypertension*. Pp 681-685.

Carswell, H.V., Dominiczak, A.F., Macrae, I.M. (2000). Estrogen status affects sensitivity to focal cerebral ischemia in the stroke-prone spontaneously

hypertensive rat. *American Journal of Physiology: Heart and Circulatory Physiology*. 278: H290-H294.

Chard, T., Hudson, C.N., Edward, C.R.W. and Boyd, N.R.H. (1971). Release of oxytocin and vasopressin by the human foetus during labour. *Nature,* 234: 352-354.

Chauhan, V., Negi, R.C., Verma, B. and Thakur, S. (2009). Transfusion Transmitted Malaria in a Non-Endemic Area. *Journal of the Association of Physicians of India*. 57:653-654.

Chukwuani, C.M. (1999). Socio-economic implication of multi-drug resistant malaria in the community: how prepared is Nigeria for this emerging problem. *West African Journal of Medicine*; 18(4): 303-306.

Chukwuocha, U.M., Osuagwa, A.E., Dozie, I.N.S., Nwoke, B.E.B., Onwuliri, C.O.E. and Ukaga, C.N. (2009). The clinical pattern and complications of severe malaria in parts of Imo River Basin of Nigeria. *Nigerian Hospital Practice*.3(6):76-79.

Chung, M.K., Han, S.S. and Roh, J.K. (1993). Synergistic embryotoxicity of combination of pyrimethamine and folic acid in rats. *Reproductive Toxicity*; 7: 463-68.

Circosta, C., Sanogo, R. and Occhiuto, F.(2001). Effect of colatropis procera on oestrous cycle and on estrogenic functionality in rats. *Farmace;* 56: 373-378.

Claessen, F.A., Van-Boxtel, C.J., Perenboom, R.M., Targe, R.A., Wetsteijn, J.C. and Kager, P.A. (1998). Quinine pharmacokinetics.Ototoxic and cardiotoxic effects in healthy Caucasian subjects and in patients with *falciparum* malaria. *Tropical Medicine and International Health*; 3(6): 482-489.

Cowman, A.F. (1995). Mechanism of drug resistance in malaria. *Australian New Zealand Journal of Medicine;* 25(6): 837-844.

Danis, M. and Gentilini, M. (1998). Malaria, a worldwide scourge. *La Revue du Practicien.*; 48(3): 254-7.

Dannenberg, A.L., Dorfman, S.E. and Johnson, J. (1983). Use of quinine for self-induced abortion. *South Medical Journal*; 76(7): 846-9.

Davidson, D. E. Jr. (1994). Role of arteether in the treatment of malaria and plans for further development. *Transactions of Royal Society of Tropical Medicine and Hygiene*; 88(suppl 1); 51-52.

De Wit, W., van Mourik, I., and Wiesenhaan, P.E.(1988). Prolonged maternal indomethacin therapy associated with oligohydramnios: case reports. *British Journal of Obstetrics and Gynaecology* 95: 303-305.

Desai, M., ter-Kuile, F.O., Nosten, F., McGready, R., Asamoa, K. Brabin, B. and Newman, R.D. (2007). Epidemiology and burden of malaria in pregnancy. *Lancet infectious diseases* 7(2): 93-104.

de-Vries, P.J. and Dien,T.K. (1996). Clinical Charmacology and Therapeutic potential of Artemisinin and its derivatives in the treatment of malaria. *Drugs*; 52(6): 818-36.

Ejiofor, J.I. (2006). Pregnancy related and neuropharmacological studies of artemether in laboratory animals. Unpublished Ph.D. Dissertation. Ahmadu Bello University Zaria, Nigeria.

Ejiofor, J.I., Kwanashie, H.O. and Anuka, J.A.(2006). Some Pregnancy- Related Effects of Artemether in Laboratory Animals. *Journal of Pharmacology*; 77(4): 166-170. (Doi:10.1159/000094521)

Erin, D.O., Resch, S.C.andGoldie,S.J. (2012). Assessing health and economic outcomes of interventions to reduce pregnancy-related mortality in Nigeria. *Biomed Central Public Health*, 12: 786.

Esamai, F., Ayuo, P., Owino-Ongor, W., Rotich, J., Ngindu, A., Obala, A., Ogaro, F., Quoqiao, L. ,Xingbo, G. and Guanggian, L. (2000). Rectal dihydroartemisinin versus intravenous quinine in the treatment of severe malaria: a randomised clinical trial. *East African Medical Journal;* 77(5): 273–278.

Fischer, P.R. (1997). Congenital malaria: an African survey. *Clinical Paediatrics*; 36(7): 411-413.

Folly, M. and Tilley, L. (1998). Quinoline antimalarials: mechanism of action and resistance and prospects for new agents. *Pharmacology and Therapeutics*; 79(1): 55-87.

Fontanet, A.L., Johnston, D. Walker, A.M., Rooney, W., Thumasarn, K., Sturchler, D., Macdonald, M., Hours, M. and Wirth, D.F. (1993). High prevalence of mefloquine-resistant falciprum malaria in eastern Thailand. *Bulletin of the World Health Organization*; 71(3-4): 377-383.

Forsling, M. (1979). The neurohypophyseal hormones. In: *Physiology and Control of Parturition in Domestic Animals.* Ellendorf, F. (ed.) Elsevier, Amstrdam.

Francesco, C., Lina, R.T., and Alberto, M. (2012). Advances in the treatment of malaria. *Mediterranean Journal of Haematology and Infectious Diseases.*4(1): 587-579.

Freeman, M.E. (1988): The ovarian cycle of the rat, physiology of reproduction. In: Knobil, E. and Neil, J. (eds.), Raven press ltd. New York. Pp. 1893-1928.

Fried, M. and Duffy, P.E. (1996). Adherence of *Plasmodium falciparum* to chondroitin sulphate A in the human placenta. *Science*; 272(5267): 1502-1504.

Gillies, M.T. and DeMeillon, B. (1968). The anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region). *The South African Institute for Medical Research*; No. 54: 343.

Goldman, J.M., Copper, R.L. and Mc Elory, W.K. (1997). Blockade ovulation in the rat by systemic and ovarian interbursal administration of fungicide sodium N – dimethyl dithiocarbonate. *Reproductive Toxicology*; 15:185-190.

Goodwin, T.M., Paul, R., Silver, H., Spellacy, W., Parsons, M., Chez, R., Hayashi, R., Valenzuela, G., Creasy, G.W. and Merriman, R. (1994). The effect of oxytocin antagonist atosiban on preterm uterine activity in the human. *American Journal of Obstetrics and Gynaecology*.170: 474-478.

Greenwood, B.M. (1997). What's new in malaria control? *Annals of Tropical Medicine and Parasitology*; 91(5): 523-531.

Gu, H.M., Warhurst, D.C. and Peters, W. (1984). Uptake of (3H) dihydroartemisinin by erythrocytes infected with *Plasmodium falciparum* in-vitro. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; 78 (2): 265-70.

Guyatt, H.L. and Snow, R.W.(2004). Impact of malaria during pregnancy on low weight in sub-Saharan Africa. *Clinical Microbiology Reviews*; 17(4): 760-9.

Health Research Extension Act of 1985 Public Law 99-158 November 20, 1985, "Animals in Research"

Hoffmann, E.T., Ronn, A.M., Lanhoff, R.J. and Bygbjeerg, I.C. (1992). Malaria and pregnancy.*UgeskLaeger*; 154(39): 2662-2665.

Holton, P. (1948). A modification of the method of Dale and Laidlaw for the standardization of posterior pituitary extract. *British Journal of Pharmacology;* 3: 328-334.

Hugh, R.K. and Barber, M.D. (1981). Symposium on Drugs in Pregnancy: Introduction. *Obstetrics and Gvnecology*; 58: 5 (supplement) 1-2.

Huszar, G. and Roberts, J. M. (1982). Biochemistry and Pharmacology of the Myometrium and Labour: Regulation at the Cellular and Molecular levels. *American Journal of Obstetrics and Gynaecology*. 142(2): 225-237.

Ismail, M.R., Ordi, J., Menendez, C., Ventura, P.J., Aponte, J.J., Kahigwa, E., Hirt, R., Cardeba, A. and Alonso, P.L. (2000). Placental pathology in malaria: a histological, immunohistochemical and quantitative study. *Human Parasitology*; 31(1): 85-93.

Jimoh, A.A.G. (2003). Epidemiological study of malarial parasites in pregnant mothers, placentae and newborns at Mongomo, Guinea Equatorial. *Nigerian Clinical Review;* 24-27.

Kager, P.A. and Wetsteyn, J.C. (1996). Malaria and drug resistance. *Netherlands TijdschrGeneeskd*; 140(3): 151-155

Karbwang, J., Sukontason, K., Rimchala, W., Namsiripongpun, W., Tin, T., Auprayoon, P., Tumsupapong, S., Bunnag, D. and Harinasuta, T. (1992). Preliminary report: a comparative clinical trial of artemether and quinine in severe *falciparum* malaria. *Southeast Asian Journal of Tropical Medicine and Public Health*; 23(4): 768-72.

Karlsson, K.K., Hellgren, U., Alvan, G. and, Rombo, L. (1990). Audiometry as a possible indicator of quinine plasma concentration during treatment of malaria. *Transactions of Royal Society of Tropical Medicine and Hygiene*. 84(6): 765–767.

Katzung,B.G. and Masters, S.B.(2011). Basic and clinical pharmacology 11th edition. Lange 796-799.

Kaye, D.K., Kakaure, O., Nakimuli, A., Osinde, M.O., Mbalinda, S.N. and Kakande, N. (2014). Lived experiences of women who developed uterine rupture following severe obstructed labour in Mulago hospital, Uganda. *Reproductive Health;* 11:31.

Kilunga-kubata, B., Eguchi.N.,Urade, Y., Yamashita, K., Mitamura, T., Tai, K., Hayaishi, O. and Horii, T. (1998). *Plasmodium falciparum* produces prostaglandins that are pyrogenic, somnogenic and immunosuppressive substances in humans. *Journal of Experimental Medicine*; 188(6): 1197-1202.

Klufio, C.A. (1992). Malaria in Pregnancy.*Papua- New Guinea Journal of Medicine;* 35(4): 249-257.

Kohen, D. (2004). Psychotropic Medication in Pregnancy. *Advances in Psychiatric Treatment;* 10: 59–66.

Kohn, D.F., and Clifford, C.B. (2002). Biology and diseases of rats.Laboratory animal medicine.2nd academic press; San Diego California.Pp 121-165.

Krungkrai, S.R. and Yuthavong, Y. (1987). The antimalarial action of *Plasmodium falciparum* of quinghaosu and artesunate in combination with agents, which

modulate oxidant stress. *Transactions of Royal Society of Tropical Medicine and Hygiene;* 81(5): 710-4.

Ksheerasagar, R.L. and Kalwal, B.B. (2008). Effects of carbosulfan administration schedules on Estrous cycle and follicular dynamics in Albino mice. Industrial Health; 46: 210-216.

Kurtis, J.D., Mtalib, R., Onyango, F.K., Duffy, P.E. (2001). Human resistance to *Plasmodium falciparum* increases during puberty and is predicted by dehydroepiandrosterone sulphate levels. *Infectious Immunology*; 69(1): 123-128.

Lawson, P.T. (2001). In: Lawson, P.T.(ed.). Genetics and breeding. Laboratory animal technician: training manual. *American Association for Laboratory Animal Science*; Memphis Tennessee.pp 27-37.

Lechner, A., Bogner, G., and Hasenohrl, G. (1997). Postpartum endomyometritis in a case of unknown tertian malaria. *Infection*; 25(3): 185-186.

Lee, K.S., Divis, P. C. S., Zakaria, S. K., Matusop, A., Julin, R. A. *et al* (2011). *Plasmodium knowlesi*: Reservoir Hosts and Tracking the Emergence in Human and Maraques PLOS *Pathogens* 7 (4).

Legros, J. J., Chiodera, P., Geenen, V., Smitz, S., and von Frenckell, R. (1984). Dose-response relationship between plasma oxytocin and cortisol and adenocorticotropin concentrations during oxytocin infusion in normal men. *Journal of Clinical Endocrinology and Metabolism.* 58: 105-109.

Leo, K.U., Grace, J.M., Li, Q., Peggins, J., Mitchell, A.L., Aguila, T. and Brewer, T.G. (1997). Effects of *Plasmodium berghei* infection on arteether metabolism and disposition. *Pharmacology*; 54:55.

Leskovac, V. and Peggins, J.O. (1992). Hepatic metabolism of artemisinin drugs: Induction of hydrogen peroxide production in rat liver microsomes by artemisinin drugs. *Compendium of Biochemistry and Physiology* C; 101(2): 203-208.

Leskovac, V. and Theoharides, A.D. (1991). Hepatic metabolism of artemisinin drugs: metabolism of arteether in rat liver cytosol. *Compendium of Biochemistry and Physiology* C; 99(3): 391-396.

Levy, G. (1981).Pharmacokinetics of Foetal and Neonatal Exposure to Drugs. *Obstetrics and Gynaecology:* 58: 5 (supplement), 9-16.

Lin, A.J., Klayman, D.L. and Milhous, W.K. (1987). Antimalarial activity of new water-soluble dihydroartemisinin derivatives. *Journal of Medical Chemistry*; 30(11): 2147-2150.

Lin, A.J., Lee, M. and Klaymam, D.L. (1989). Antimalarial activity of new water-soluble dihydroartemisinin derivatives; stereospecificity of ether side-chain. *Journal of Medical Chemistry*; 32(6): 1249-1252.

Lin, A.J., Li, L.Q., Klaymam, D.L., George, C.F. and Flippen-Anderson, J.L. (1990).Antimalarial activity of new water-soluble dihydroartemisinin derivatives (3).Aromatic amine analogs. *Journal of Medical Chemistry*; 33(a): 2610-2614.

Lindsay, S.W., Ewald, J.A., Samung, Y., Apiwathnasorn, C. and Nosten, F. (1998).Thanaka (Limoniaacidissima) and deet (di-methyl benzamide) mixture as a mosquito repellent for use by Karen women. *Medical Veterinary Entomology:* 2(3): 295-301.

Long, J.A. and Evan, I.I.M. (1922): The estrus cycle in rat and its associated phenomena. Memories of University of California 6: 1-148.

Looareesuwan, S., Phillips, R.E., White, N.J., Kietinun, S., Karbwang, J., Rackow, C., Turner, R.C. and Warrell, D.A. (1985). Quinine and severe *falciparum* malaria in late pregnancy. *Lancet.*; 2(8445): 4–8.

Looareesuwan, S., Viranvan, C., Vanijanonta, S., Wilairatana, P., Suntharasamai, P., Charoenlarp, P., Arnold, K., Kyle, D., Canfield, C. and Webster, K. (1992). Randomized trial of artesunate and mefloquine alone and in sequence for acute uncomplicated *falciparum* malaria. *Lancet*; 339(8797): 821-4.

Lu, J.K.H, Anzolone, C.R and Lapolt, P.S.(1994). Relationship of Neuroendocrine function to reproductive decline during aging in the female rat. *Neurobiology of Aging*. 15: 541-544.

Luzzi, G.A. and Peto, T.E. (1993). Adverse effects of antimalarials. An update *Drug Safety;* 8: 4295-4311.

Maeda, K.I., Ohkura. S., and Tsukamura, H. (2000). Physiology of reproduction. In:krinke G.J. (ed). *The Laboratory Rat.* Academic press; San Diego, California.pp145-176.

Marsh, K. (1999). *The management of severe malaria*. In: Malaria and Infectious Diseases in Africa. Smithkline Beecham Pharmaceuticals; No. 9(3): 127-129.

Matteelli, J.T., Caligaris, S., Castelli, F. and Carosi, G. (1997). The placenta and malaria. *Annuals of Tropical Medicine and Parasitology*; 91(7): 803-810.

Mc-Gready, R. and Nosten, F. (1999). The thai- burmese border: drug studies of *Plasmodium falciparum* in pregnancy. *Annals of Tropical Medicine and Parasitology*; 93(suppl1): S19-S23.

McGregor, I.A. (1984). Epidemiology, malaria and pregnancy. *American Journal of Tropical Medicine and Hygiene*; 33: 517-525.

McGuinness, D., Koram, K., Bennett, S., Wagner, G., Nkrumah, F. and Riley, E. (1998). Clinical case definitions' for malaria: clinical densities in African infants. *Transactions of Royal Society of Tropical Medicine and Hygiene;* 92(5): 527-31.

Meshnick, S.R. (1998). Artemisinin antimalarials: mechanism of action and resistance. *Tropical Medicine*; 58(3 suppl): 13-17.

Molineaux, L. and Gramiccia, G. (1980). The Garki Project: Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa. WHO; Geneva.

Montes, G.S. and Luque, E.H. (1988). Effects of ovarian steroids on vaginal smear in the rat. *Acta Anatomica* 133: 192-199.

Muentener, P., Schlagenhauf, P. and Steffen, R. (1999). Imported malaria (1985-95): trends and perspectives. *Bulletin of the World Health Organization*. 77: 560-566.

Murphy, S.C. and Breman, J.G. (2001). Gaps in the childhood malaria burden in africa: cerebral malaria, neurological sequelae, anaemia, respiratory distress, hypoglycaemia and complications of pregnancy. *American Journal of Tropical Medicine and Hygiene;* 64(1-2 suppl): 57-67.

Mutabingwa, T.K., Malle, L.N., Verhave, J.P., Eling, V.M., Meuwissen, J.H. and de-Guea, A. (1993).Malaria chemosuppression during pregnancy.Its effect on the newborn's passive malaria immunity. *Journal of Tropical Medicine and Hygiene and Geography;* 45(4): 150-156.

Mvondo, J.L., James, M.A. and Cambell, C.C. (1992). Malaria and pregnancy in cameroonian women. Effect of pregnancy on *Plasmodium falciparum* parasitaemia and the response to chloroquine. *Tropical Medicine and Parasitology*; 43(1): 1-5.

Myint, P.T., Shwe, T., Soe, L., Htut, Y. and Myint, W. (1989). Clinic study of the treatment of cerebral malaria with artemether (qinghaosu derivatives).*Transactions of Royal Society of Tropical Medicine and Hygiene;* 83:72.

Na-Bangchang, K., Ubalee, R., Thanavibul, A. and Saenglertsilapachai, S. (2000). Absence of significant pharmacokinetic and pharmacodynamics interactions between artemether and quinolone antimalarials. *European Journal of Drug Metabolism and Pharmacokinetics*; 25(3-4): 171-178.

Naing, R.D., Win, U.H., New, D.Y., Myint, U.P.T. and Shwe, U.T. (1988). The combined use of artemether, sulfadoxine and pyrimethamine in the treatment of uncomplicated *falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; 82(4): 530-531.

Navaratnam, V., Manson, S.M., Sit, N.W., Grace, J., Li, Q. and Alliaro, P. (2000). Pharmacokinetics of artemisinin-type compounds. *Clinical Pharmacokinetics*; 39(4): 255-270.

Nequin, L.G., Alvarez, J., Schwartz, N.B., (1979). Measurement of serum steroid and gonadotrophin levels and uterine and ovarian variable throughout 4-day and 5-day estrus cycles in the rat. *Biology of Reproduction*; 20(3): 659-670.

Nicholas, X., Granier, H., Martin, J. and Klotz, F. (2000). Epidemiology and therapeutic aspects of plasmodial infection from *Plasmodium vivax*. *Journal of Tropical Medicine* (mars); 60(4): 402-408.

Nosten, F., McGready, R. and d'Alessandro, U. (2006). Antimalarial drugs in pregnancy: a review *Current Drug Safety*; 1(1): 1-5.

Nosten, F., Vincent, M., Simpson, J., Yei, P., Thwai, K.L., de-Vries,A., Chongsuphajaisiddhi, T. and white, N.J. (1999). The effects of mefloquine treatment in pregnancy. *Clinical Infectious Disease*; 28(4): 808-815.

Nwaigwe, C.I., Adegunloye, B.J. and Sofola, O.A. (1997). Effect of chloroquine on the contractility of the smooth muscles of the rat uterus, trachea and urinary bladder. *Journal of Basic and Clinical Physiology and Pharmacology;* 8(4): 279-286.

Nwanjo, H.U. Iroagba, I.I., Nnatuanya,I.N. and Eze, N.A. (2007). Anti-fertility activity of dihydroartemisinin in male albino rats. *The Internet Journal of Endocrinology*; 4(1).

OECD guideline part 5; preparation, reading and reporting of vaginal smears. http://www.oecd.org/chemicalsafety/testing/43754782.pdf

Okitolonda, W., Delacollette, C., Malengreau,,M., and Henquin, J.C. (1987). High incidence of hypoglycaemia in African patients treated with intravenous quinine for severe malaria. *British Medical Journal* (Clinical Research Ed.); 295(6600): 716–718.

Onigbogi, O., Ajayi, A. A., Ukponmwan, O. E., (2000). Mechanism of chloroquine body- scratching behaviour in rats: evidence of involvement of endogenous opioid peptides. *Pharmacological Biochemistry and Behaviour*, 65(2): 333-337.

Oyedeji, K.O., Bolarinwa, A.F. and Afolabi, O.A. (2013). Effect of tetracycline on Haematological and reproductive parameters in female albino rats. *Asian journal of Pharmaceutical and clinical Research;* 6(suppl. 2): 225-229

Pan, H.Z., Lin, F.B. and Zhang, Z.A. (1989). Effect of sodium artesunate on malaria infected human erythrocytes. *Proceedings of China Academic Medical Science Peking Union Medical College*; 4(4): 181-185.

Pandey, A.V., Tekwani, B.L., Singh, R.L. and Chautan, V.S. (1999). Artemisinin, an endoperoxide antimalarial disrupts the haemoglobin catabolism and hemedetoxication systems in malarial parasites. *Journal of Biology and Chemistry*; 274:27.

Peter,V., Den, E., Hong, N.V., Indra,V., Thang, N.D., Le, X.H., Hung, N.M., Jozef, A., Umberto, D. and Anne, E. (2009). Human *Plasmodium knowlesi* Infections in Young Children in Central Vietnam, *Malaria Journal*, 8:249.

Phaneuf, S., Rodinguez, L.B., Tambyraja, R.L., Mackenzie, I.Z. and Lopez, B.A. (2000). Loss of Myometrial oxytocin receptors during oxytocin – induced and oxytocin augmented labour. *Journal of Reproductive Fertility;* 120(1): 91-7.

Philip-Howard, P. A. and ter-Kuile, F. O. (1995). CNS adverse events associated with antimalarial agents. Facts or Fiction. *Drug safety*; 12(6) 370-383.

Phillips-Howard, P.A. and Wood, D. (1996). The safety of antimalarial drugs in pregnancy. *Drug safety*; 14:131-145.

Phillips-Howard, P.A., Robert, S., Steffen, L.K., Bart, V., Jannie, S., Elizabeth, F. and Ralph, E. (1998). Safety of mefloquine and other antimalarial in the first trimester of pregnancy. In: Penelope, A.(ed.) *Journal of Travelling Medicine*; 5:121-126.

Price, R.N., Vav-Vugt, M., Phaipun, L., Luxemberg, C., Simpson, J., McGready, R., ter-kuile, F., Kham, A., Chongsuphajaisiddhi, T. and White, N.J. (1999). Adverse effects in patients with acute *falciparum* malaria treated with artemisinin derivatives. *American Journal of Tropical Medicine and Hygiene*; 60(4):547-555.

Ramu, K. and Baker, J.K. (1997). Identification of the glucoronides of the hydroxylated metabolites of the antimalarial arteether in rat plasma and urine by thermospray high- performance liquid chromatography/ mass specremeetry. *Journal of Pharmaceutical Sciences*; 86(8): 915-20.

Ribeiro, I.R. and Olliaro, P. (1998). Safety of artemisinin and its derivatives. A review of published and unpublished clinical trials. *Journal of Tropical Medicine (mars)*; 58(3suppl): 50- 3.

Sachs, J, and malaney, P. (2002).The economic and social burden of malaria. *Nature*, 415:680-685.

Salako, L.A. and Sowunmi A (1992). Disposition of quinine in plasma, red blood cells and saliva after oral and intravenous administration to healthy adult Africans. *European Journal of Clinical Pharmacology*.1992; 42(2): 171–174.

Salako, l. (1998). Artemisinin and its derivatives: the regulatory and policy implications for African countries. *Tropical Medicine*; 58(3suppl) 82-84.

Salako, L.A. (1999). Malaria control priorities and constraints. *La Parasitologia*; 41(1-3): 495-6.

Santiso, R. (1997). Effects of chronic parasitosis on women's health. *Journal of internal Gynaecology and Obstetrics*; 58(1): 129-136.

Sharanabasappa, A., Vjaykumar, B. and Sararaah,B.P. (2002). Effect of *momordica charantia* seed extracts on ovarian and uterine activities in albino rats. *Pharmaceutical biology;* 40(7): 501-507.

Shukla, K.L., Gund, T.M. and Meshnick, S.R. (1995). Molecular modelling, studies of the artemisinin (quinghaosu)-hemin interaction: docking between the antimalarial agent and its putative receptor. *South East Asian Journal of Tropical Medicine and Public Health*; 29(2): 225-7.

Shuler, A.V. (1985).''*Malaria, Meeting the Global Challenge,''* Agency for International Development. Oelgeschlager, Gunn and Hain, Boston Massachusetts,110.

Shulman, C.E. (1999). Malaria in pregnancy: Its relevance to safe motherhood programmes. *Annals of Tropical Medicine and Parasitology;* 93 suppl: 59-66.

Shwe, T., Myint, P.T., Myint, W., Htut, Y., Soe, L. and Thew, M. (1989). Clinical studies on the treatment of cereberal malaria with artemether and mefloquine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; 83(4): 489.

Singh, N., Shukla, M.M. and Sharma, V.P. (1999). Epidemiology of malaria in pregnancy in central India. *Bulletin of World Health Organization*; 77(7): 567-572.

Skinner, T.S., Manning, L.S., Johnston, W.A. and Davis, T.M. (1996). In-vitro stage specific sensitivity of *Plasmodium falciparum* to quinine and artemisinin drugs. *Journal of Internal Parasitology*; 26: 519-529.

Slinger, R., Giulivi, A., Bodie-Collins, M., Hindieh, F., St. John, R., Sher, G., Goldman, M., Ricketts, M. and K. C. Kain (2001). Transfusion-transmitted malaria in Canada. *Canadian Medical Association Journal*. 164(3):377-379

Smith, M.S., Freeman, M.E. and Neil, J.D. (1975). The control of progesterone secretion during the Oestrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. *Endocrinology, 96*(1): 219-226.

Spornitz, U. M., Rinderknecht, B.P., Edelmann, A., Scheidegger, B. and Cairoli, F. (1994): Ultrastructure as a basis for dating of rat endometrium. *Anatomical Record*. *238*(2): 163-176.

Steketee, R.W., Nahlan, B.L., Parise, M.E. and Merendez, C. (2001). The burden of malaria in pregnancy in malaria endemic areas. *American Journal of Tropical Medicine and Hygiene;* 64(1-2 suppl.): 38-35.

Stuebe, A. (2012). Treatment of preterm labour: *tocolytics.*

Subramanian, D., Moise, K. J.Jr. and White, A.C.Jr. (1992). Imported malaria in pregnancy: report of four cases and review of management. *Journal of Clinical Infectious Diseases*; 15: 408-13.

Sylvia, J.S., Alan, J.K., Julie, W., Baktiar, O.K., David, L.H., Patricia, D.H., and Stephanie, J.M. (2005). Lack of correlation of vaginal impedance measurement with hormone levels in the rat. Contemporary topics in laboratory animal science; 44(6): 37-42.

Taha, T.T. and Gray, R.H. (1993). Malaria and perinatal mortality in central Sudan. *American Journal of Epidemiology*; 138(8); 563-8.

Taylor, W.R., Widjaja, H., Richie, T.L., Basri, H., Orht, C., Taufik, E., Jones, T.R., Kain, K.C. and Hoffman, S.L. (2001).Chloroquine/doxycycline combination versus chloroquine alone and doxycycline alone for the treatment of *Plasmodium falciparum* and *Plasmodium vivax* malaria in north-eastern irianjaya, Indonesia. *American Journal of Tropical Medicine and Hygiene*; 64(5-6): 223-8.

Tracy, J.W. and Webster Jr. L.T. (2001). Chemotherapy of Parasitic Infection, In: Hardman, J.G., Limbard, L.E. and Gilman, A.G. (eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th Ediion, Mc Graw-Hill, New-York; 1021-73.

Valecha, N., Bhatia, S., Mehta,S., Biswas, S. and Dash, A. P (2007). Congenital malaria with atypical presentation: A case report from low transmission area in India. *Malaria Journal.* 6:43

van-Breemen, C., and Saida, K. (1989). Cellular Mechanism Regulating (ca2+) in Smooth Muscle. *Annual Review of Physiology*.51: 315-329.

Veinot, J. P., Mai, K.T. and Zarychanski, R. (1998). Chloroquine related cardiac toxicity. *Journal of Rheumatology*; 25(6): 1221-5

Wagner, G., Koram, K., McGuinness, D., Benneth, S., Nkrumah, F. and Riley, E. (1998). High incidence of asymptomatic malaria infections in a birth cohort children less than one year of age in Ghana, detected by multicopy gene polymerase chain reaction. *American Journal of Medical Hygiene*; 59(1): 115-123.

Walmer, D.K., Wrona, M.A., Hughes, C.L. and Nelson, K.G. (1992). Lactoferrin expression in the mouse reproductive tract during the natural estrous cycle:

correlation with circulating estradiol and progesterone. *Endocrinology;* 131: 1458-1466.

Ward, S.E., Sevene, E.J.P., Hastings, M.I., Nosten, F. and McGready, R. (2007). Antimalarial drugs and pregnancy: safety, pharmacokinetics, and pharmacovigilance. *Lancet infectious diseases* 7: 136-144.

Weiss, G. (1987). The production and function of ovarian relaxin. In: *the Primate Ovary*. Stouffer, R.L. (ed.) Plenum Press, New York; pp. 223-236.

Wernsdorfer, W.H. (1994). Epidermiology of drug resistance in malaria *Acta Tropica;* 56: 143-56.

White, N. J. (2008). *Plasmodium knowlesi*: The Fifth Human Malaria Parasite. *Clinical infectious diseases.*46: 172-173.

White, N.J. (1996). The treatment of malaria. *New England Journal of Medicine*; 335: 800–806.

White, N.J. and Alliaro, P. (1998). Artemisinin and derivatives in the treatment of uncomplicated malaria. *Tropical Medicine*; 58(3 suppl): 54-6.

White, N.J. and Pukrittayakamee, S. (1993). Clinical malaria in the tropics. *Australian Journal of Medicine*; 159(3): 197-203.

WHO (2012). The 2011 World Malaria Report.

WHO and UNICEF (2003). African Malaria Report, Chapter 3: Prompt and Effective Treatment pp. 31-37.

WHO, (2009). Global antimalarial drug policies database - WHO African region. September edition.

WHO, (2010). Malaria Treatment Guidelines.

WHO, (2013). Malaria Entomology.

WHO, (1998). *Life in the 21st century: a vision for all.* The World Health Report; Geneva; 53.

WHO. (1987). *Biology of Malaria*. Technical Report Services No. 734:56.

Wilairatna, P., Kructsood, S., Silachamroon, U., Singhaswanon, P., Vannaphan, S., Faithong, S., Klabprasit, M., Bangchang, S.N., Olliaro, P. and Looareesuwan, S. (2000). Clinical trial of sequential treatments of moderately severe and severe malaria with dihydroartemisinin suppository followed by mefloquine in Thailand. *American Journal of Tropical Medicine and Hygiene*; 63(5-6): 290-4.

Wilson, J.T. (1975). Pragmatic assessment of medicines available for young children and pregnant or breast-feeding women. In: Morselli, P.L., Garttini, S. and Sereni, F. (Eds.) Basic and Therapeutic Aspects of Perinatal Pharmacology. Raven Press, New York, U.S.A; pp.411-21.

www.malariasite.com/malaria/combinations.htm.

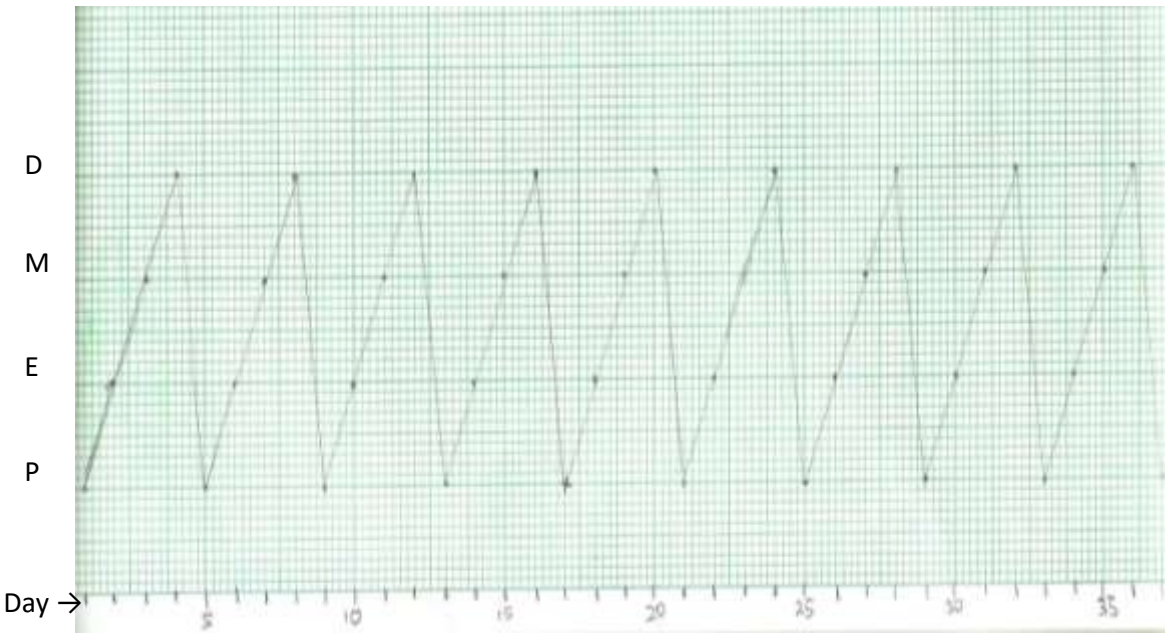
YakoubAdenAbdi, O.E., Gustafsson Lars, L., Ericsson, O., Urban, H. (1995). Handbook of Drugs for Tropical Parasitic Infections.2: 1995.

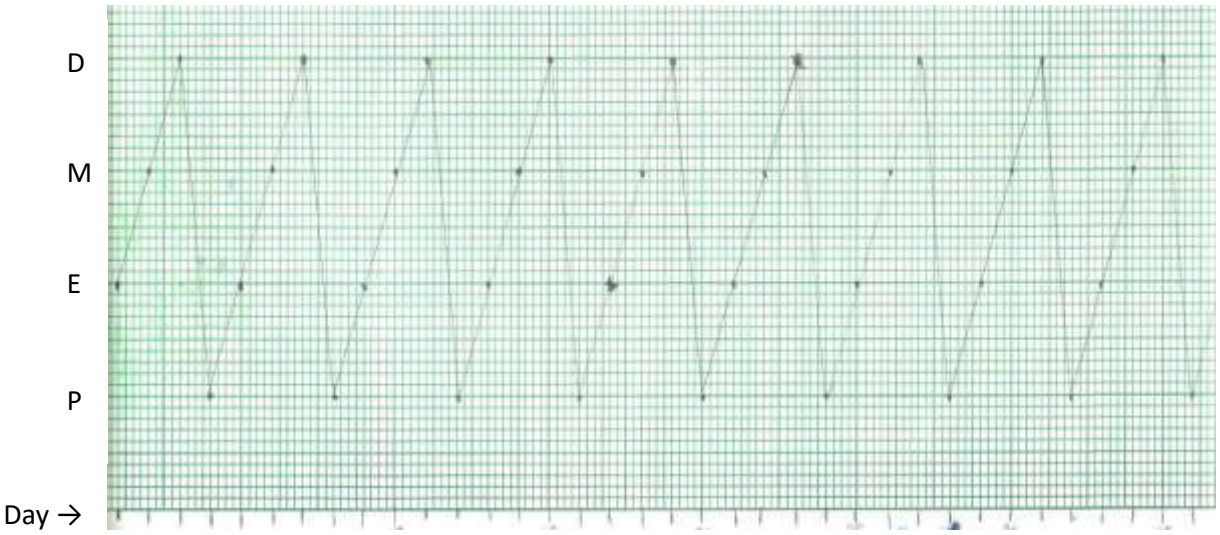
Yang, D.M. and Liew, F.Y. (1993). Effects of quinghaosu (artemisinin) and its derivatives on experimental cutaneous leishmaniasis. *Parasitology*; 106(1): 7-11.

Young, W.C., Boling, J.L. and Blandau, R. (1941). The vaginal smear picture, sexual receptivity and time of ovulation in the albino rat. *Anatomical Records*; 80: 37-45.

Zhao, Y., Hanton, W.K., Lee, K.H. (1986). Antimalarial agents, artesunate, an inhibitor of cytochrome oxidase activity in *Plasmodium berghei*. *Journal of Natural Product*; 49: 139-42.

APPENDIX I





Upper Panel: 1a Distilled water in which chloroquine, quinine and sulphadoxine/ pyrimethamine was dissolved

Lower panel: 1b 3% v/v Tween 80 in which artemether was suspended

Before=4 cycles (day 1-16)
During= varied (maximum 8 days, day 17- 24)
After= 4 cycles after antimalarial administration (up to day 40 max.)

APPENDIX II

Daily smear obtained for rats before, during and artemether administration

Day → / Rat ↓	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	M	D	M	D	E	M	D	M	M	M	M	M	M	M	M	M	M	M	M	D	P
2	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	M	D	E	P	E	M	P	E	M	P	P	P	P	E	M	D	P
3	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	M	M	M	D	M	D	P	P	P	E	M	M	D	P	E	M	D	P
4	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	M	D	M	M	M	M	D	M	D	P	E	E	M	M	M	M	P	E	E	M	D	P
5	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	P	P	M	M	P	E	M	M	M	E	P	E	M	D	P	E	M	D	P	E
6	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	E	M	D	P	E	E	P	P	P	P	P	E	D	P	E

P= proestrus, E= oestrus, D= dioestrus and M= metoestrus

APPENDIX III

Number of oestrous cycle stages in rats before, during and after artemether administration

STAGES	BEFORE	DURING (Actual)	DURING (Corrected)	AFTER
P	4..00± 0.00	1.33 ± 0.38*	3.05 ± 0.88	4.50 ± 0.87
E	4.00± 0.00	0.83 ± 0.28**	1.90 ± 0.64*	3.50 ± 0.81
M	4.00± 0.00	3.16 ± 0.36	7.24 ± 0.84*	5.50 ± 1.52
D	4.00 ± 0.00	1.66 ± 0.19***	3.81 ± 0.44	2.50 ± 0.31**

During A: Artemether (32 mg/kg loading dose & 16mg /kg maintenance dose), i.p. daily for 7days

During C: corrected for 16- days utilized for before and after artemether administration

P=proestrus, E= oestrus, M= metoestrus, D= dioestrus

n=6 rats, Bar= mean ±SEM, *= $p<0.05$, **=$p<0.01$, ***=$p<0.001$

APPENDIX IV

Daily smear obtained for rats before, during and chloroquine administration

Day → / Rat ↓	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	D	P	E	M	D	M	D	P	E	M	D	P	E	M	D	P
2	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	P	P	P	M	P	E	E	M	D	D	P	E	M	D	P	E	M
3	M	**D**	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	D	D	P	E	D	E	P	M	D	E	M	P	E	M	D	P
4	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	D	P	E	P	M	D	P	P	E	M	D	P	E	M
5	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	D	E	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E
6	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	M	D	D	P	P	M	D	P	E	M	D	P	E	M	D	P	E

P= proestrus, E= oestrus, D= dioestrus and M= metoestrus

APPENDIX V

Number of oestrous cycle stages in rats before, during and after chloroquine administration

STAGES	BEFORE	DURING (Actual)	DURING (Corrected)	AFTER
P	4.00± 0.00	0.66 ± 0.19*	5.33 ± 1.54	4.50 ± 0.20
E	4.00± 0.00	1.16 ± 0.15**	1.33 ± 1.22*	3.66 ± 0.30
M	4.00± 0.00	0.66 ± 0.19*	5.33 ± 1.54	3.50 ± 0.20
D	4.00± 0.00	0.50 ± 0.20*	4.00 ± 1.63	4.33 ± 0.30

During A: Chloroquine (2.5 mg/kg [max.25 mg/ kg] dose) i.p daily for 2 days

During C: corrected for 16- days utilized for before and after Chloroquine administration

P=proestrus, E= oestrus, M= metoestrus, D= dioestrus
n=6 rats, Bar= mean ±SEM, *= p<0.05, **=p<0.01, ***=p<0.001

APPENDIX VI

Daily smear obtained for rats before, during and quinine administration

Day→ / Rat ↓	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	M	D	M	P	P	D	M	M	P	E	E	E	E	E	E	P	E	M	D	P	E
2	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	M	M	M	P	E	D	D	d	d	d	d	d	d	d	d	d	d	d	d	d
3	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	D	D	M	M	P	M	M	M	D	D	P	M	M	P	M	M	P	E	M	D	P
4	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	D	D	M	M	P	D	D	D	P	E	P	E	M	D	D	P	E	M	D	P	E
5	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	P	M	M	M	M	M	M	M	M	P	D	P	E	M	D	P	E	M	D	P	E	M	D
6	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	M	M	P	M	M	P	D	D	P	E	M	M	D	P	E	M	D	P	E	M	D	P

P= proestrus, E= oestrus, D= dioestrus and M= metoestrus; d= death

APPENDIX VII

Number of oestrous cycle stages in rats before, during and after quinine administration

STAGES	BEFORE	DURING (Actual).	DURING (Corrected).	AFTER
P	4.00± 0.00	2.16 ± 0.28*	4.33 ± 0.56	3.16 ± 0.60
E	4.00± 0.00	0.16 ± 0.15***	0.33 ± 0.30***	3.16 ± 1.04
M	4.00± 0.00	4.16 ± 0.54	8.33 ± 1.09**	3.66 ± 1.02
D	4.00± 0.00	1.50 ± 0.39**	3.00 ± 0.78	3.83 ± 0.44

During A: Quinine (20 mg/kg loading dose & 10 mg /kg 8 hourly) i.p. daily for 7 days

During C: corrected for 16- days utilized for before and after Chloroquine administration

P=proestrus, E= oestrus, M= metoestrus, D= dioestrus
n=6 rats, Bar= mean ±SEM, *= p<0.05, **=p<0.01, ***=p<0.001

APPENDIX VIII

Daily smear obtained for rats before, during and sulphadoxine/pyrimethamine administration

Day → / Rat ↓	1	2	3	4	5		6	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	M	P	E	E	M	E	P	E	M	D	D	P	E	M	D
2	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	M	D	P	M	M	P	E	M	D	P	E	M	D	P	E	M
3	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	M	P	E	M	D	P	E	M	D	P	E	M	D	P
4	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	P	M	D	P	E	M	P	P
5	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	P	P	P	P	E	M	D	P	E	M	D	P	E
6	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	D	P	E	M	D	P	E	M	D	P	E	M	D	P

P= proestrus, E= oestrus, D= dioestrus and M= metoestrus

APPENDIX IX

Number of oestrous cycle stages in rats before, during and after Sulphadoxine/Pyrimethamine administration

STAGES	BEFORE	DURING (Actual).	DURING (Corrected).	AFTER
P	4.00± 0.00	0.50 ± 0.20	8.00 ± 3.26	4.66 ± 0.45
E	4.00± 0.00	0.16 ± 0.15	2.66 ± 0.99	3.66 ± 0.30
M	4.00± 0.00	0.16 ± 0.15	2.66 ± 0.99	4.16 ± 0.50
D	4.00± 0.00	0.16 ± 0.15	2.66 ± 0.99	3.50 ± 0.31

During A: Sulphadoxine/Pyrimethamine (25 mg/kg/1.25 mg/kg) i.p. once
During C: corrected for 16- days utilized for before and after SP
n=6 rats, Bar = mean ± SEM, No statistical significance

APPENDIX X

Proestrous stage for all antimalarials

STAGES	BEFORE	DURING (Actual)	DURING (Corrected)	AFTER
Artemether	4.00± 0.00	1.33 ± 0.38***	3.05 ±0.88	4.50 ± 0.87
Chloroquine	4.00± 0.00	0.66 ± 0.19***	5.33 ± 1.54	4.50 ± 0.20
Quinine	4.00 ± 0.00	2.16 ± 0.28**	4.33 ± 0.56	3.16 ± 0.60
Sulphadoxine/Pyrimethamine	4.00 ± 0.00	0.50 ± 0.20**	8.00 ± 3.26	4.66 ± 0.45

Actual data refers to the period of antimalarial administration
Corrected for 16- days utilized for before and after antimalarial administration
n=6 rats, Bar = ± SEM

APPENDIX XI

Oestrous stage for all antimalarials

STAGES	BEFORE	DURING (Actual)	DURING (Corrected)	AFTER
Artemether	4.00± 0.00	0.83 ± 0.38***	1.90 ±0.64 **	3.50 ± 0.81
Chloroquine	4.00± 0.00	0.16 ± 0.15***	1.33 ± 1.22	3.66 ± 0.30
Quinine	4.00 ± 0.00	0.16 ± 0.15***	0.33 ± 0.30***	3.16 ± 1.04
Sulphadoxine/Pyrimethamine	4.00 ± 0.00	0.16 ± 0.15***	2.66 ± 0.99	3.66 ± 0.30

Actual data refers to the period of antimalarial administration
Corrected for 16- days utilized for before and after antimalarial administration
n=6 rats, Bar = ± SEM

APPENDIX XII

Metoestrous stages for all antimalarials

STAGES	BEFORE	DURING (Actual)	DURING (Corrected)	AFTER
Artemether	4.00± 0.00	3.16 ± 0.36	7.24 ±0.84	5.50 ± 1.52
Chloroquine	4.00± 0.00	0.66 ± 0.19***	5.33 ± 1.54	3.50 ± 0.20
Quinine	4.00 ± 0.00	4.16 ± 0.54	8.33 ± 1.09	3.66 ± 1.02
Sulphadoxine/Pyrimethamine	4.00 ± 0.00	0.16 ± 0.15***	2.66 ± 0.99	4.16 ± 0.50

Actual data refers to the period of antimalarial administration
Corrected for 16- days utilized for before and after antimalarial administration
n=6 rats, Bar = ± SEM

APPENDIX XIII

Dioestrous stages for all antimalarials

STAGES	BEFORE	DURING (Actual)	DURING (Corrected)	AFTER
Artemether	4.00± 0.00	1.66 ± 0.19**	3.81 ±0.44	2.50 ± 0.31
Chloroquine	4.00± 0.00	0.50 ± 0.20***	4.00 ± 1.63	4.33 ± 0.30
Quinine	4.00 ± 0.00	1.50 ± 0.39**	3.00 ± 0.86	3.83 ± 0.44
Sulphadoxine/Pyrimethamine	4.00 ± 0.00	0.16 ± 0.15***	2.66 ± 0.99	3.50 ± 0.31

Actual data refers to the period of antimalarial administration
Corrected for 16- days utilized for before and after antimalarial administration
n=6 rats, Bar = ± SEM

APPENDIX XIV

SUMMARY OF DE JALON PHYSIOLOGICAL SOLUTION

QUANTITY FOR 10 LITRES

Nacl	90g
Kcl	42ml
D- Glucose	15g
$NaHCO_3$	5g
$Cacl_2$ (MOLAR)	2.7ml
Aerating gas	95%O_2+5% CO_2
Water	10 litres

List of abbreviations

µg/ml= Microgram per millilitre.

ACT= Artemisinin combination therapy.

ANOVA=Analysis of variance.

AT= Artemether.

CNS= Central nervous system.

CQ= Chloroquine.

DHA= Dihydroartemisinin.

FSH= Follicle stimulating hormone.

GIT= Gastro intestinal tract.

GnRH= Gonadotrophin releasing hormone.

LH= Lutenizing hormone.

mg /ml= Milligram per millilitre.

ng /ml= Nanogram per millilitre.

QN= Quinine.

RBC= Red blood cells.

SEM= Standard error of mean.

SP = Sulphadoxine /pyrimethamine.

ADH= Antidiuretic hormone.

ACTH= Adrenocorticotrophic hormone.

NADPH= Nicotinamide adenine dinucleotide phosphate.