EFFECTS OF 1, 4-NAPHTHOQUINONE ON MALE REPRODUCTIVE FUNCTIONS IN MICE

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A THESIS IN THE DEPARTMENT OF PHYSIOLOGY

SUBMITTED TO THE

FACULTY OF BASIC MEDICAL SCIENCES,
COLLEGE OF MEDICINE

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY (PH.D)

OF THE UNIVERSITY OF IBADAN

FEBRUARY, 2014

ABSTRACT

The derivative of naphthoquinone, 1, 4-naphthtoquinone, has been extensively shown to be cytotoxic, with significant antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory and antipyretic properties. Many cytotoxic and antibacterial agents have been implicated in male reproductive disorders. However, information on the effects of 1, 4-naphthoquinone on male reproductive functions is scanty. This study was carried out to assess the effects and probable mechanisms of action of 1, 4-naphthoquinone on male reproductive functions in mice.

Swiss albino male mice (18-22 g, 20 per group) were treated with either Tween 20 (vehicle, control) or 1, 4-naphthoquinone (0.1, 0.5, 1 and 2 mg/kg) intraperitoneally (i.p) for 5 days/week for 2 weeks. Another set of male mice (10 per group) were treated with chloroquine (5, 10, 15 and 20 mg/kg, i.p) to serve as standard drug. At the end of the experimental period, epididymal sperm motility, viability and count were determined by microscopy. Serum from 4 mL of blood per mouse was analysed for testosterone, Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Prolactin and cortisol by Enzyme Immunoassay (EIA) technique. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Superoxide Dismutase (SOD), catalase, malondialdehyde and blood haemoglobin (Hb) levels were measured spectrophotometrically. Red and White Blood Cells (RBC and WBC) counts were measured using haemocytometry while Packed Cell Volume (PCV) was determined using micro haematocrit reader. Testicular histology was done using heamatoxylin and eosin stain. Data were analysed using ANOVA and Student's t-test at $p = 0.05$.

The 1, 4-naphthoquinone (2 mg/kg) significantly decreased sperm motility (70.3 ± 1.5 vs 41.3 ± 8.3%), viability (88.3 ± 4.7 vs 63.0 ± 2.7%) and count (20.8 ± 2.5 vs 9.4 ± 3.0 million/mL) relative to controls. Similarly, chloroquine (20mg/kg) significantly decreased sperm motility

(72.3 ± 2.1 vs 42.0 ± 2.0 %), viability (87.3 ± 4.0 vs 64.3 ± 4.0%) and count (21.2 ± 2.6 vs 9.8 ± 0.9 million/mL). There was a significant decrease in testosterone (4.8 ± 0.7 vs 2.4 ± 0.4 ng/mL) and LH (4.3 ± 0.2 vs 2.6 ± 0.6 ng/mL) levels in mice treated with 1, 4-naphthoquinone (2 mg/kg) when compared with the controls. However, prolactin (1.6 ± 0.4 vs 2.7 ± 0.5 ng/mL), FSH (2.8 ± 0.9 vs 3.2 ± 0.4ng/mL) and cortisol (1.0 ± 0.0 vs 3.1 ± 0.2 ng/mL) significantly increased in 1, 4-naphthoquinone (2 mg/kg) and chloroquine-treated groups. Administration of 1, 4-naphthoquinone decreased all haematological indices except granulocytes while chloroquine decreased RBC, WBC, Hb and PCV. Treatment with 1, 4-naphthoquinone significantly increased ALT (115.7 ± 20.0 vs 134.9 ± 9.4 IU/L), AST (632.5 ± 70.6 vs 667.3± 62.6 IU/L), SOD (2.6 ± 0.8 vs 4.5 ± 0.4 IU/L) and catalase (78.3 ± 4.8 vs 96.2 ± 4.0 IU/L) but decreased malondialdehyde (0.42 ±0.02 vs 0.21 ± 0.02 units/mg protein). The 1, 4-naphthoquinone caused greater seminiferous tubules damage than chloroquine.

The 1, 4-naphthoquinone altered parameters of male reproductive functions in mice. These effects were not mediated via oxidative stress mechanism.

Key words: 1, 4-naphthoquinone, male reproductive hormones, antioxidant enzymes

Word count: 486

DEDICATION

This thesis is dedicated to JEHOVAH GOD, the Giver and Provider of life and on whom we should show absolute dependence and to my late father; Chief Honourable Sunday Etukudoh and my Late mother; Deaconess IquoUdo who though barely literate saw education as the foundation for future greatness and lastly to my wife and children to serve as a source of inspiration to them.

ACKNOWLEDGEMENTS

My profound and sincere thanks go to my main supervisor, Professor (Mrs) Adeyombo F. Bolarinwa for her dedication and commitment towards the completion of this thesis. I appreciate your great and untiring role in mentoring me during the course of this programme. I am short of words but only to emphasize that I Cherish your meticulousness and attention to details. Your strict observation of ethical principles in this course of this study has manifested in the quality of this work. May God bless you abundantly.

I wish to also appreciate Professor Yinusa Raji for the pains taken to go through this thesis. I remain grateful to you for never turning me down irrespective of your busy schedule. Your steadfastness and selfless contribution to the smooth completion of this work would remain indelible in my memory.

I like to appreciate my Head of Department Prof A. A. Fasanmade for his encouragement, advice and support during the course of my programme. I am thankful to him for laying the foundation of this work when you supervised my M.Sc dissertation. I value those opportunities when we usually had very useful academic exchanges. I cannot fail to appreciate your kindness and fatherly disposition.

I cannot fail to acknowledge Dr. S. B. Olaleye, Sub-Dean Postgraduate of the Faculty of Basic Medical Sciences for his contribution towards the course of this work. Your encouragement and friendly disposition was a great tonic that helped me immensely. I am grateful for the good times we shared together.

It is my pleasure to remember Prof A. R. A. Alada for his unflinching support, encouragement and valuable contributions. I wish to appreciate Dr (Mrs) Elsie O. Adewoye and Dr F. S.

Oluwole for being great motivators. I wish to thank my friend; Dr. S. A. Onasanwo for his usual kind words of encouragement. I like to thank Mr Okon and the other Technical staff in the Department of Physiology who were quite helpful during the analyses of my samples.

My gratitude also go to my staff in the Federal School of Medical Laboratory Science, Jos particularly Mr. Frederick Olowu, Mr Ernest Demtoe, Miss Susan Umoh and Bola Deko for their support in the course of this work. I wish to also remember my friends particularly Prof M. F. Useh of the Department of Medical Laboratory Science, University of Calabar, Calabar and Dr B. E. Bassey of the World Health Organisation for their support. The contributions of my friends namely Iyke Isiguzoro, Dr James Damen, Dr Okwori, Prince Joshua Adetunji, Dr James Adisa is hereby acknowledged.

I wish to deeply appreciate my dear wife; Mrs Maria Etukudoh for constantly reminding, challenging and motivating me to ensure that I take the work to a smooth conclusion. Thanks for the maturity and patience you showed particularly during those long hours of my experimentation. I wish to appreciate my lovely children; Sifon, Nkereuwem Jnr, Uyai, Mkpouto and Etimbuk for their understanding. I like to appreciate all my kindred in the family of Late Chief Udo Etukudoh for their prayers and support.

Nkereuwem Sunday **Etukudoh**

CERTIFICATION

I certify that Mr. Nkereuwem Sunday **ETUKUDOH,** carried out this work titled "**Effects of 1, 4-Naphthoquinone on Male Reproductive Functions in Mice"** under my supervision in the Department of Physiology, College of Medicine, University of Ibadan, Nigeria.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

Naphthoquinones are a novel class of compounds with potent antimalarial activity against *Plasmodium falciparum*. Malaria is a devastating disease, which affects many people in the world and accounts for about two million deaths annually (W. H. O 2001). Malaria infection starts in the mammalian host with the injection of plasmodium sporozoites by a mosquito bite. Sporozoites travel to the liver where they cross the sinusoidal wall through Kupffer cells and then migrate through several hepatocytes before they establish an infection with the formation of a parasitophorus vacuole within the vacuole. The sporozoites develop and generate millions of merozoites that are released into the blood stream. With the infection of erythrocytes, the clinical phase of malaria begins (Margarida, *et al,* 2006).

Plasmodium berghei was probably first described in 1946 by Vincke in blood films of the stomach contents of *Anopheles dureni*. In 1948, it was subsequently found in blood films of *Grammony surduster* collected in Kinsanga, Katanga (Butcher and Beetsma, 2001). Although the natural vector of *Plasmodium berghei* is Anopheles mosquito. Malaria is a major tropical disease which kills almost two million people annually. The population at risk from this disease has increased because of the difficulties in eradicating the mosquito vectors in the endemic regions and emergence and spread of the parasite resistance to all commonly used anti-malaria (Govind, 2002).

Since anti –malaria are the major arsenal for the treatment of the disease, there is an urgent need for the introduction of newer drugs with novel mechanisms of action which will be effective against all strains of the parasite (Govind, 2002). Among these drugs are the

naphthoqunones and their derivatives which have proved effective with powerful anti- malaria activity against plasmodium falciparum (Govind, 2002). A derivative of naphthoquinones is 2 – amino-3-chloro-1, 4- naphthoquinone which is the most potent and more effective anti-malaria agent than commonly used chloroquine.

In addition several antimalarial drugs are available for treatment of malaria, their efficacy has been limited by rapid development of resistant strains of malarial parasites, especially *Plasmodium falciparum*, during the last 30years (White, 1999). An escalation of the incidence of malaria –related morbidity and mortality appears inevitable in view of the increase and world-wide spread of the drug resistant parasite (Trape *et al*, 1998). This is a catastrophe for poor tropical countries, which cannot afford more expensive alternative antimalarial drugs. Except for the recently introduced artemisinin and its analog (Meshnick and Taylor, 1996), most of the currently used antimalarials are ineffective against the malaria parasites, which acquire or develop multidrug resistance.

Chloroquine used to be the first drug of choice against malarial in endemic areas for more than three decades until the emergence and spread of chloroquine resistant strains of *P. falciparum*, which reduced its effectiveness in virtually all part of the world. Chloroquine however remains the treatment of choice for falciparum malaria in most parts of Africa (Sowunmi *et al*, 1998). This is due to its ready availability, tolerability and affordability. Two such drug-resistant *Plasmodium* strains are the Indochina III / CDC, or W2 strain. The W2 strain is resistant to chloroquine, pyrimethamine and sulfadoxine but susceptible to mefloquine, while the D6 strain is resistant only to chloroquine.

Recently, atovaquone, a hydroxyl naphthoquinone derivative, has been shown to have antimalarial activity (Hudson, 1993; Kapadia *et al*, 2001). It is also a potent antimalarial drug

effective against the multidrug-resistant malaria parasite (Gay *et al*, 1997), without any *invitro* evidence of cross-resistance (Basco *et al*, 1995). This discovery opened a new approach for design of antimalarials.

Rodents, like primates, birds and reptiles are possible host of malaria. At present, eleven species of plasmodium have been described in population of tree dwelling rodents which live in the central African rainforest and many of these have been widely studied as different experimental models of human malaria (Cox, 1988, Landau and Gauret, 1998). In an experimental model involving cogenic mice and *Plasmodium chabaudi*, adult mice have a mild crisis that rapidly resolves into a long lasting chronic infection similar to infection described in wild host (Cox, 1988, Landau and Gauret, 1998). Nevertheless, despite the apparently low virulence of the infection, several pathologies have been reported in infected male and female rats up to several months after parasitic clearance (Cox, 1988; Gauret et al, 1988).

It is pertinent to note that many antimalarial drugs have been implicated in male infertility. There is paucity of information in the literature regarding the mechanism of the antifertility effects of antimalarials investigated so far. For instance, mention has been made of the apparent reduction in fertility in male rats by chloroquine (Sairam, 1978). This drug has been reported to completely obliterate Leydig cell response to leutropin and hormones having leutropin – like activity *invitro* (Sairam 1978) and also inhibition of basal and stimulated testosterone secretion in decapsulated testis *invitro* (Nduka, 1986). Adeeko and Dada (1998) also reported a remarkable reduction in fertilizing capacity of epididymal sperm in rats treated with human therapeutic doses of chloroquine. However, this study did not report the mechanism underlying this action of chloroquine. Quinine has also been used as an abortifacient for many years while instillation of high concentrations of quinacrine dihydrochloride in the uterus

prevents implantation in rats (Joseph and Kincl, 1974) and causes tubal occlusion in women (Davidson, 1973). It is also important to note that the action of antimalarial agents on male reproduction is not limited to synthetic drugs. The effects of *Azadirachta* extract on male reproductive functions have been reported (Prakash et al, 1988). Raji and Bolarinwa (1997), Parveen et al, (2003) have also reported the antispermatogenic and antisteroidogenic actions of *Quassia amara* plant extract in rats. Both *Azadirachta indi*ca and *Quassia amara* extracts are used in traditional medicine to treat malarial and *Quassia* extract antimalarial activity has been reported to be potent against chloroquine resistant strains of *Plasmodium falciparum* (Polonsky, 1986).

In view of the growing interest in the development of Naphthoquione into antimalarial drugs, it is important to investigate its probable effects on reproductive functions. The chemical structures of naphthoquinones and chloroquine are similar. The present study was therefore designed to investigate the effects of naphthoquinones and chloroquine on male reproductive functions in parasitized and non-parasitized albino mice.

Specific Objectives:

1. To determine the individual effects on male reproductive functions of chloroquine and naphthoquinone alone in normal and parasitized mice.
2. To determine the mechanisms of action of chloroquine and naphthoquinone on male reproductive functions with reference to:
    a. Effects of naphthoquinone and chloroquine on temperature changes in Plasmodium infected and normal uninfected mice.
    b. Haematological variables in chloroquine and naphthoquinone treated male mice.

c. Histopathology and photomicrograph of the various organs such as the testis, epididymis.

d. Effects of chloroquine and naphthoquinone on serum antioxidant enzymes such as catalase, superoxide dismutase (SOD) and malondialdehyde (MDA).

e. Effects on spermatogenesis

3. To determine and validate the effects of *Plasmodium berghei* on male reproductive functions in mice for the purpose of the present study.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 PHYSIOLOGY OF MALE REPRODUCTIVE SYSTEM

The reproductive system is a network of organs within an organism which works together for the purpose of reproduction. The male reproductive system is made up of the pair of gonads called the testicles, the vas deferens, the seminal vesicle, the prostate glands and the penis. The testis is anatomically and biochemically equipped to perform two functions; spermatogenesis and androgen biosynthesis. The testis is composed of loops of convoluted seminiferous tubules and it is located in the scrotum which is suspended outside the abdominal cavity. Along the inside walls of the seminiferous tubules are two basic cell types; Sertoli cell and Leydig cell. The Sertoli cells are also found inside the seminiferous tubules and are involved in the maturation of spermatozoa. Outside the seminiferous tubules are located the Leydig cell in which androgen synthesis takes place. Both ends of the convoluted tubules drain into the epididymis, where further maturation of the spermatozoa takes place and from here the spermatozoa pass into the vas deferens before they enter the body of the prostrate glands.

A pair of seminal vesicle is located on each side of the prostate glands which produce mucus like nutritive secretion that is rich in fructose and other chemicals. Prostatic ducts carrying a milky alkaline secretion from other accessory glands such as Cowper's glands are added and this suspension of spermatozoa known as semen is emptied into the ejaculatory duct.

2.1.1 SPERMATOGENESIS

The hypothalamus produces a decapeptide called gonadotrophic releasing hormone (GnRH), which travels through the hypophyseal portal venous system to the adeno-hypophysis (anterior pituitary) where it enhances release of two polypeptide hormones namely

luteinizinghormone (LH) and follicle stimulating hormone (FSH). Beginning in early adolescence, the spermatogonia develop into primary spermatocytes, which undergoes meiotic cell divisions to reduce the chromosome number from diploid to haploid number.

Further development of the primary spermatocytes into secondary spermatocytes and then into spermatids is under the control of testosterone, the predominant androgen synthesized in the testis. Further maturation of spermatids into spermatozoa, which takes place in the deep fold of the cytoplasm of the Sertoli cells, is under the control of FSH. Both FSH and testosterone stimulate the synthesis of androgen binding protein (ABP), the Sertoli cells mediate the first meiotic division of spermatocytes. The epididymal cells produce nutrients and factors necessary for the final maturation process of the spermatozoa, which include the ability to fertilize an ovum. This phenomenon is called capacitation. Peak levels of spermatogenesis take place about 2^0C lower than the temperature of the body, hence the location of testes outside the body. It should be noted that spermatogenesis does not follow a cyclic hormonal pattern. The whole of spermatogenesis takes approximately 6 weeks and at completion, the spermatozoa are released from the Sertoli cells into the lumen of the seminiferous tubules (Alex *et al*, 2006)

2.1.2 ACCESSORY GLANDS OF MALE REPRODUCTIVE SYSTEM

The accessory glands of the male reproductive system are the seminal vesicles, the prostate glands and the bulbourethral glands. They produce a combination of fluid produced during ejaculation. It is believed that 95% of the entire ejaculate originates from the accessory glands while only 5% comes from the testes (Robert *et al*, 1990).

TESTES

The size of the testes in relation to the mice body weight varies within individual mice. However, within mammals, the size increases when the mating habits are more polygamous than

monogamous. With rats, the testes are larger due to their polygamous mating habit (Simeons, 2005). The side of the glands facing each other is where the coagulation glands are located. Each one of these glands has a duct that joins with the vas deferens to form an ejaculating duct that empties into the uretha (Simeons, 2005)

SEMINAL VESICLES

The seminal vesicles are paired highly convoluted pyriform glands. Each vesicle is about 5 to 6cm long and 1 to 2cm wide. They lie on each side of the midline, lateral to the ampula of the vas deferens, posterior to the urinary bladder and superior to the prostate. Each gland is closed at its apex, open at its lower extremities and coiled (Mannand, 1981).

The proteinase inhibitor secreted by the seminal vesicles stabilizes the sperm acrosomal membrane (Mannand, 1981). The secretions of the seminal vesicles are viscid, slightly yellowish in colour and alkaline in PH. About 70% of human ejaculate originates from seminal vesicles (Mannand, 1981).

COWPER'S GLANDS

The Cowpers glands also known as the bulbourethral glands are made up of multiple lobules that secrete a pre- ejaculatory or Cowpers fluid, into the urethra during sexual arousal. This secretion serves to clear urine from the urethra and to lubricate it and the vagina. The product may also serve as an energy source for the sperm (Simeons, 2005).

PREPUTIAL GLANDS

These are small sebaceous exocrine glands (glands that do not secrete directly into the bloods stream). The preputial glands are located towards the end of the prepuce (the sheath that covers the penis) that secretes smegma. Smegma is composed of oils, moisture and shed epithelial cells. It moisturizes the glands and in a healthy rat or mice has an anti-bacterial and

antiviral property. Smegma can however form a proteinacious plug, otherwise known as penis plug that can interfere with urination and may lead to infection if not removed on time (Simeon, 2005).

PROSTATE GLANDS

The prostate gland is a dense gland located behind and slightly below the urinary bladder and is partially wrapped around the penis. The prostate gland produces most of the fluid found in semen as well as the fluid that transports the sperm through sperm ducts to the urethra. This fluid is released through the penis during ejaculation (Simeons, 2005).

The prostate secretes 0.5 – 1.5ml of a thick milky fluid (15 – 30% of the ejaculate volume) which is usually slightly alkaline (pH 7.2), although it has been reported to be slightly acidic (pH 6.8), (Hamilton, 1976). The variation in pH is probably due to the level of the citric acid in the fluid. Human prostatic fluid contains a number of constituents. The best known is acid phosphatase, citric acid, polyamines and bivalent cations (Ca^{2+}, Zn^{2+}, Mg^{2+}, Na^{+} and K^{+}). In addition, lamellated acidophilic bodies called corporal amulcea can be found in the fluid. In this case, secretory granules and fragment of the cells components including cytoplasm, small organelles and endoplasmic reticulum are also secreted (Hamilton, 1976).

2.1.3 MALE REPRODUCTIVE HORMONES

During childhood, the sexual features usually undeveloped. When an individual reaches about 14 years of age, conspicuous changes take place that lead towards sexual maturity and the ability to reproduce. This time known as puberty is when the characteristics that differentiate the sexes gradually appear. In males the beard and body hair begin to grow, the shoulders are broad, the voice becomes deeper pitched and more attention is paid to the opposite sex. In females, the

mammary glands and hips enlarge, subcutaneous fat is deposited, the reproductive tract begins a series of cyclic changes and the attention of young men becomes of interest.

At the start of puberty, the hypothalamus which is the portion of the brain nearest to the pituitary gland greatly increases its secretion of a hormone called gonadotropin releasing factor. This growth-stimulating hormone (GSH) acts on the anterior lobe of the pituitary gland stimulating the gland to secrete the gonadotropic hormones-follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones, in turn act on the gonads, (sex glands) the testes in males and ovaries in females. If pituitary is experimentally removed, the reproductive organs remain infantile, the individual is sterile and no sexual cycle occurs (Engelking, 2000).

Under the influence of FSH and LH, the gonads grow and begin to secrete large amount of sex hormones. The male sex hormones, including testosterone and androsterone, are called androgens. Androgens cause the male sex organ to mature and stimulate sexual behaviours. The reproductive activities of male mammals are under hormonal control. The endocrine of the testis responsible for secondary sexual characters is the testosterone evidently produced by the Leydig cells or interstitial cells between the seminiferous tubules. If this hormone is injected into a castrated individual, the accessory sex organs enlarge, the secondary sexual characters develop and the behaviour becomes that of a normal (uncastrated animal). Spermatogenesis is controlled by the hormone, testosterone.

Male reproductive hormones come from the hypothalamus; the anterior pituitary gland, and the testes. In males, luteinizing hormone and follicle stimulating hormone facilitate spermatogenesis. The steroid hormone testosterone is secreted from the testes and can be detected in early embroyonic development up until puberty. At puberty, rising levels of

testosterone stimulate male reproductive development including secondary characteristics(Burnstein, 2002).

TESTOSTERONE

Testosterone is a steroid from the androgen group. In mammals, testosterone is primarily secreted in the testes of males and the ovaries of females, although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid.

In men, testosterone plays a key role in health and well being as well as preventing osteoporosis (Dabbs, 2000).Testosterone is secreted by the Leydig cells which lie in the interstitial tissue of the testes between the semiferous tubules. Its production is stimulated by luteinizing hormone and it in turn inhibits luteinizing hormone secretion by negative feedback.

Testosterone is involved in sexual differentiation, development of secondary sexual characteristics, spermatogenesis and anabolism. In the male, the effect of testosterone depends on intracellular conversion to the even more potent androgen, dihydrotestosterone by the enzyme 5α-reductase in target cells.

Testosterone and to a lesser extent oestradiol circulate bound to a carrier protein, sex hormone binding globulin (SHBG) as well as albumin. As with other hormones only the free fraction is metabolically active.

LUTEINIZING HORMONE (LH)

Luteinizing hormone (LH) is a glycoprotein gonadotropic hormone secreted by the anterior pituitary gland. It is released by the anterior pituitary in hourly pulses called “Circhoral oscillations”. Luteinizing hormone binds to receptors in the testes in males and the ovaries in females and regulates gonadal function by promoting sex steroid production and gametogenesis i.e the process by which spermatozoa and ova are formed.

The role of luteinizing hormone is slightly different in men and women. In men, luteinizing hormone stimulates testosterone production from the interstitial cells of the testes (Leydig cells). In both sexes, luteinizing hormone stimulates testosterone production from the interstitial cells of the testes (Leydig cells). Luteinizing hormone is also essential for the maturation of spermatozoa. In the testes, luteinizing hormone binds to receptors on Leydig cells stimulating synthesis and secretion of testosterone. The cells in the ovary respond to luteinizing hormone stimulation by secretion of testosterone which is converted into estrogen by adjacent granulosa cells.

FOLLICLE STIMULATING HORMONE (FSH)

Follicle stimulating hormone is a glycoprotein gonadotropic hormone secreted by the anterior pituitary gland. Follicle stimulating hormone bind to receptors in the testes (in males) and the ovaries (in female) to regulate gonadal function by promoting sex steroid production and "gametogenesis" that is the process by which spermatozoa and ova are formed. Follicle stimulating hormone stimulates testicular growth and enhances the production of a protein that causes high local concentrations of testosterone near the sperm – which is an essential factor in the development of normal spermatogenesis. Follicle stimulating hormone is essential for the maturation of spermatozoa.

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) are called gonadotropins because they stimulate the gonads (in males, the testes, and in female, the ovaries). They are not necessary for life, but are essential for reproduction. These two hormones are secreted from cells in the anterior pituitary called gonadotrophs. Most gonadotrophs secretes only LH or FSH, but some appear to secrete both hormones. Physiologic effects of the

gonadotropin are known only in the ovaries and testes; together they regulate many aspects of gonadal functions in both males and females.

The principal regulator of LH and FSH secretion is gonadotropin releasing hormone (GnRH also known as LH releasing hormone). GnRH is a ten amino acid peptide that is synthesized and secreted from hypothalamic neurons and binds to receptors on gonadotrophs. As depicted in the figure above, GnRH stimulates secretion of LH, which in turn stimulates gonadal secretion of the sex steroids testosterone, estrogen and progesterone. In a classical negative feedback loop, sex steroids inhibit secretion of GnRH and also appear to have direct negative effects on gonadotrophs. This regulatory loop leads to pulsatile secretion of luteinizing hormones and to a much lesser extent, FSH. The number of pulses of GnRH and LH varies from a few per day to one or more per hour. In females, pulse frequency is clearly related to stage of the cycle (Bowen, 2004).

In the diseased state, diminished secretion of LH or FSH can result in failure of gonadal function (hypogonadism). This condition is typically manifest in males as failure in production of normal numbers of sperm. In females, cessation of reproductive cycles is commonly observed. Elevated blood levels of gonadotrophics usually reflect lack of steroids negative feedback. Removal of the gonads from either males or females as is commonly done to animals, leads to persistent elevation in LH ad FSH. In humans, excessive secretion of FSH and/or LH most commonly is the result of gonadal failure or pituitary tumours. In general, elevated levels of gonadotropins have no biological effect.

Studies have shown that hyperprolactinaemic male rats exhibited deficits in copulatory behaviour and this can be reversed by treatment with systematically administered GnRH that can stimulate copulatory behaviour independent of plasma testosterone levels. Gonadectomized,

pituitary-grafted adult male rats given a single injection of 500ng GnRH or saline pituitary-grafted adult male rats bearing capsule of testosterone were administered a single injection of 500ng GnRH or saline pituitary-grafted castrates displayed copulatory deficits, relative to sham-operated castrates with replacement. The effects of administration of a LHR analogue (ICI-118,630) on plasma concentration of hormones and weights of reproductive organs were studied in male mice. A single injection of the analogue (154μ/kg body weight) caused a transient elevation of the concentration of LH in the plasma up to 7 times the level in control mice. Daily administration of the analogue for a period of 3 weeks caused a small but significant decrease of the weight of the ventral prostate. The weight of the seminal vesicles in mice treated with the analogue, however, did not differ from that in control animals. The concentration of testosterone and LH in the plasma of these mice was not significantly different from those in control mice. It is demonstrated that the LHRH- analogue has no castration-like effect on the accessory sex organs of the sex organs of the male mouse, which is therefore not a suitable model in the study of compounds.

Raji *et al*, (2006) reported a progressive duration dependent decrease in sperm motility, sperm count and viability in parasitized male albino mice infected with 15, 30 and 45% *Plasmodium berghei*. They also reported that, there were significant decrease in serum testosterone and increase in cortisol levels in the infected mice compared with the controls. There was also a progressive decrease in red blood cell count and packed cell volume, and there was a progressive increase in white blood cell count and spleen and liver weight. They also reported that, there was no significant change in weight of the testis and epididymis. The overall results suggest that, the malaria parasite could depress the male fertility indices. Rudali *et al*, (1974) and Bronson *et al*, (1989) also reported that there is modification in the testosterone level

in a mice infected with *Plasmodium chabaudi* resulting from the erythrocytic proliferation of the parasite.

In another development, Petit *et al*, (2004) reported in experimental host-parasite association involving BALB male mice infected with *Plasmodium chabaudi* to investigate the influence of the parasite on the sexual physiology and behaviours of the infected mice, that infected males displayed complete courtship behaviour leading to ejaculations and also revealed that there was a two fold decrease in the testosterone level at post injection and during recrudescence. The imbalance was also accompanied by a decrease in the overall duration of the social investigation contacts occurring during courtship and by a reduction in fertilization rate of the infected animals. They concluded that, the physiological perturbations can be regarded as an adaptive response of the host (mice) to the recrudescing parasites which illustrate the rodents capability for regulating the testosterone profiles needed to balance the competing demands of immunity and reproduction.

In the study undertaken by Bartke *et al*, (1987) to test the hypothesis that, that deficit in copulatory behaviour of a male rat may be related to the reduction in hypothalamic release of LH showed that plasma testosterone levels was significantly elevated following either dose of LHRH and the resulting LHRH can restore normal copulatory performance in hyperprolactinemic male rat and support the hypothalamic LRHR release is responsible for the behavioural deficit observed in those animals.

2.2 NAPHTHOQUINONE

2.2.1 HISTORY OF NAPHTHOQUINONE

Napthoquinones isolated from the wood of trees of the families *Bignoniaceae* and *Verbenaceae* have been subjected to an interdisciplinary study since the seventies, when Benjamin Gilbert, at the Federal University of Rio de Janeiro launched a programme on the chemistry of natural products active against endemic disease (Gilbert *et al*, 1977). The bioactive naphthquinones found include lapachol that is isolated from the heart wood of Bignoniaceae and Verbanaceae families, abundant in tropical rain forest (Gilbert *et al*, 1987).

In Brazil more than forty six types of such woods popularly known by the name *Tabebuia* species have been described. Lapachol, alpha-Lapachone and beta-Lapachone have been studied by a group since seventies, when Gilbert began a research on the chemistry of naphthoquinones isolated from *Tabeuia* species that show their activity against *Schistosoma mansoni* and *Trypanosome cruzi*. That was the starting point of their research programme on the use of quinines from the Brazilian flora against endemic diseases in Brazil (Gilbet *et al*, 1992).

Naphthoquinone or more precisely 1, 4-naphthoquinone is an organic compound. It forms yellow tricyclic crystals and has an odour similar to benzoquinone (Qian *et al*, 2001).Naphthoquinone forms the central chemical structure of many natural compounds, most notably the K vitamin (Qian et al: 2001). 1,2-nahthoquinone is a polycyclic aromatic ketone. It is a reactive metabolite of naphthalene. This compound is a quinone found in diesel exhaust particles. The accumulation of this toxic metabolite in rats from doses of naphthalene has been shown to cause eye damage, including the formation of cataracts (Qian *et al*, 2001).

The molecular structures of naphthoquinone endow them with redox properties which confer activity in various biological oxidative processes (Monks *et al*, 1992).Naphthoquinone is

sparingly soluble in cold water, slightly soluble in petroleum ether, and freely soluble in most polar organic solvents. In alkaline solutions it produces a reddish-brown colour. Because of their aromatic stability, 1, 4-naphthoquinone derivatives are known to possess anti-bacterial and anti-tumor properties. It's molecular formular is $C_6H_6O_2$ with a molar mass of 158.15g/mol and density of 1.422g/cm^3. The other properties include, melting point of 126^{0}C with boiling point which begins and subline at 100^{0}C (Merek *et al*, 2007).In folk medicine, often originating among native Ameridian populations, plants containing naphthoquinone have been employed for the treatment of a number of diseases including cancer. The toxicity and therapeutic activities of these compounds involve the formation of oxygen reactive species (Monks *et al*, 1992).

The biological redox cycles of quinones can be initiated by one election reduction leading to the formation of semiquinones unstable intermediates that react rapidly with molecular oxygen generating free radicals, which causes damage to biological membranes and interferes with biosynthetic pathways. Another alternative is the reduction of quinones by two electrons leading to the formation of endogenous hydroquinols, mediated by diphorase. This enzyme is known to promote the redox cycling of 2-hdroxy-1, 4-naphthoquinone.

This process is less harmful to the cells, and sulfatation or glucoronidation leads to inactive conjugated products which are easily eliminated. This latter pathway is considered a detoxification route for toxic quinones (Munday, 2008). Naphthoquinone derivatives are potential therapeutic agents which are under investigation. However, some such compounds are known to be toxic to both animals and humans. Many derivatives of napthoquinone are heamolytic agents, while others cause necrosis of tubular epithelial cells (Wiley, 2007).

Other names of napthoquinone are 1, 4-naphthoquinone, α- naphthoquinone among others with the common names as naphthoquinones, naphthazarins and naphthalenediones. The

O- naphthoquinone derivative is a potent inhibitor but its therapeutic potential is compromised by a short plasma half-life (22 minutes) and extremely poor oral bioavailability (less than 2%) (Pharm-Res, 1990).

The indolequinone compound E09 has good pharmacodynamic properties in terms of bioreductive activation and selectivity for either NAD(P)H: quinone oxidoreductase-1 (NQO1)-rich aerobic or NQO1-deficiently hopoxic cells. However, its pharmacokinetic properties are poor and this fact is believed to be a major reason for EO9's lack of clinical efficacy (Philips *et al*, 2004).

Naphthoquinone has the following physical properties Molecular formula $C_{10}H_60_2$.

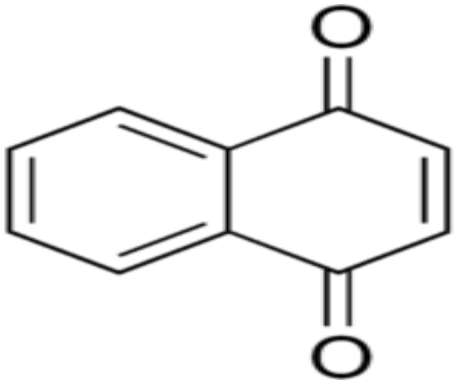

Figure 1: Structure of Naphthoquinone

Melting point 277.O^{o}F (NTP, 1992)

Vapour Density! 5.46

Specific gravity: 1.422

Boiling point begins to sublime at 100^{o}C

Molecular weight: 158.16

Water solubility: 1mg/mL 70^{o}F

The fire hazard of this compound is that it is combustible. When this compound is heated to decomposition, it emits toxic fumes and smoke. The best storage condition of this substance is to protect it from light and the container is kept tightly closed under an inert atmosphere and store under refrigerated temperature (Bock *et al*, 1976).

2.2.2 PHARMACOLOGY OF NAPHTHOQUINONE

It has now been found that certain substituted Naphthoquinones exhibit high activity against *Plasmodiumfalciparum*. Certain of these compounds exhibit activity which is compared to that of sulfadoxine.

Several substituted 2-amino-3-chloro-1, 4- naphthoquinone were tested and the most effective of these compounds was found to be 2-amino-3- chloro-1,4- naphthoquinone. The activity of this compound against W_2*P. falciparum* (strain of malaria parasites) is compared to that of sulfadoxine. The IC_{50} against the chloroquine, pyrimethamine and sulfadoxine resistant W_2 strain of *P. falciparum* was 37.3ng/ml and the IC_{90} against this strain was 83.4ng/ml. The IC_{50} value of 2-amino-3-chloro-1,4- naphthoquinone against the W_2 strain is considerably lower than that of chloroquine, pyrimethamine and sulfadoxine resistant W_2strain *P. falciparum* was 37.3ng/ml and the IC_{90} against this was 83.4ng/ml. The IC_{50} value of 2-amino-3-chloro-1,4-naphthoquinone is considerably lower than that of chloroquine, pyrimethamine and sulfadoxine, indicating that 2-amino-3-chloro-1,4- naphthoquinone has the potential to be a useful addition to the medical practitioner's arsenal of drugs effective against drug-resistant strain of *P. falciparum*.

2.3 CHEMICAL STRUCTURE OF CHLOROQUINE

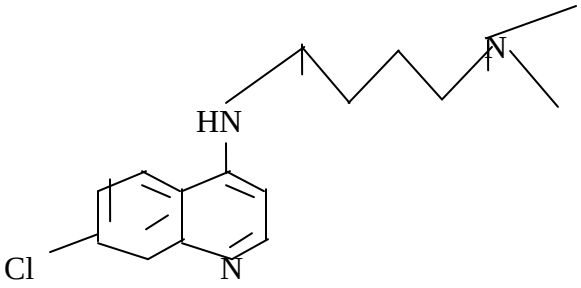

Figure 2:N- (7 chloroquinolin -4-y1) – N, N diethyl- pentane – 1,4-diaminne.

Chloroquine is a 4-aminoquinoline drug used in the treatment or prevention of malaria. Chloroquine has a very high volume of distribution as it diffuses into the body's adipose tissue. Chloroquine and its related quinines have been associated with cases of retinal toxicity, particularly when provided at higher doses for longer time. Accumulation of the drug may result in deposits that can lead to blurred vision and blindness. Chloroquine can be used for preventing malaria from *Plasmodium vivax, Plasmodium ovale,* and *Plasmodium malariae*. Many areas of the world have wide spread strain of chloroquine resistant *Plasmodium falciparum,* so other antimalarial like mefloquine or atovaquone may also be advisable (CDC,2001). Apart from its antimalarial activity, chloroquine is useful in the treatment of gastro-intestinal ameobiasis, systemic lupus erythematosis, fluke infection, discoid lupus erythemasis, rheumatoid arthritis and intrahepahtic amoebiasis rheumatois arthritis which is recently reported to have broad spectrum anti-HIV activity(Savarino *et al,* 2001). Chloroquine may cause anti-fertility in female rats(Okanlawon and Ashiru,1997),and the anti-fertility in male rats may result from reduction in the circulating plasma testosterone (Okanlawon *et al*, 1990, Freeko *et al*,1992). The Sertoli cells, seminiferous tubules, interstitial cells also provide support for germinal cells to synthesize follicle stimulating hormone receptors, androgen receptors and acts as a blood-testis barrier (Goslar *et al*, 1982). It is therefore germane to study the testicular tissue status during chronic chloroquine ingestion as it binds avidly to tissues.

2.3.1 PHARMACOKINETICS

Chloroquine is rapidly and almost completely absorbed from the gastrointestinal tract following oral administration and peak plasma concentrations of the drug are generally attained within 1-2 hours. Chloroquine is widely distributed into body tissues such as the eyes, heart, kidneys, liver and lungs, where retention is prolonged. Concentrations are two to five times

higher in erythrocytes than in intestinal wall. Chloroquine crosses the placenta and is distributed into breast milk.

Approximately 55% of the drug in the plasma is bound to plasma proteins. It is metabolized in the liver to active de-ethylated metabolites. Principal metabolite is de-ethylchloroquine. The plasma half life of chloroquine in healthy individuals is generally reported to be 72-120 hours (Savarino et al, 2003). Chloroquine is eliminated by renal route. Forty two per cent to 47% of chloroquine is excreted unchanged in the urine, 7 to 12% de

De-ethylchoroquine is excreted very slowly and may persist in urine for months or years after medication is discontinued. Urine acidification increases renal excretion by 20 to 90%.

Haemodialysis increases the clearance of chloroquine, however due to chloroquine's large volume of distribution, haemodialysis may not remove appreciable amounts in an over dose (Davidson *et al*, 2008).

2.3.2 ADVERSE EFFECT OF CHLOROQUINE

At the doses used for prevention of malaria, side effects include gastrointestinal problems such as stomach ache, itching, headache, and blurred vision. Chloroquine-induced itching is very common among black Africans (70%), but much less common in other races. It increases with age, and is so severe as to stop compliance with drug therapy. It is increased during malaria fever, its severity correlates to the malaria parasite load in the blood. There is evidence that it has a genetic basis and is related to chloroquine action with opiate receptors centrally or peripherally (Ajayi, 2000, Savarino *et al*, 2006).

When doses are extended over a number of months it is important to watch out for a slow onset of "changes in mood" (i.e depression, anxiety). These may be more pronounced with

higher doses used for treatment. Chloroquine tablets have an unpleasant metallic taste (Ajayi, 2000).

A serious side-effect is also a rare toxicity in the eye (generally with chronic use), and requires regular monitoring even when symptom free (Yam, 2000). The daily safe maximum doses for eye toxicity can be computed from one's height and weight. The use of chloroquine has also been associated with the development of central serious retinopathy (Savarino,2000).

Chloroquine is very dangerous in overdose. It is rapidly absorbed from the gut. In 1961, studies were published showing that three children who took overdoses died within 2½ hours of taking the drug. While the amount of the overdose was not cited, it is known that the therapeutic index for chloroquine is small (Cann, 1961).

According to "Plus One Journal" and cited by scientific American, an over use of chloroquine treatment has led to the development of a specific strain of *E. coli* that is now resistant to the powerful antibiotic ciprofloxacin (Davidson *et al*, 2008).

2.3.3 CHLOROQUINE RESISTANCE

Chloroquine resistance among plasmodia has been slow in developing. However, *P. falciparum* has acquired significant resistance and resistant strains have been prevalent especially in eastern America. Some of these have also acquired resistance to proguanil, pyrimithamine and mepacrine (multi drug resistance strain). Because *falciparum* produces the more severe forms of malaria with considerable mortality, emergence of such strains is the biggest threat to the antimalaria programs, and in the focus of attention for current research efforts. The mechanism of resistance in *P. falciparum*is to accumulate chloroquine (Jaypee,2003).

2.3.4 PHARMACODYNAMICS

Chloroquine and its 4 amino-quinolone congeners block the enzymatic synthesis of DNA and RNA. Chloroquine is an excellent blood schizonticide and on sporozoites. It inhibits the erythrocytic stage of development of plasmodia. Thus relapses may occur after cessation of the treatment (Ajayi, 2000).

2.3.5 INHIBITION OF THE ACTIVATION OF HUMAN COMPLEMENT AND COAGUALTION SYSTEMS BY CHLOROQUINE

The effect of chloroquine on the complement and coagulation systems has bee studied. The activation of the classical pathway of complement by antibody coated sheep erythrocytes and aggregated 1gG was blocked by chloroquine (Manco-Johnson, 2000). Similarly, chloroquine has been reported to prevent normal rabbit erythrocytes and Zymosan from activating the alternative pathway of complement. The activation of C3 by immune complexes of different solubilities was inhibited by chloroquine. Chloroquine abrogated the clotting of plasma by calcium chloride and thrombin. This effect can be reversed by the addition of excess thrombin but not of calcium chloride (Ramanathan *et al*, 2002). Chloroquine was also found to enhance degradation of FXIII in fibroblasts (Elizabeth *et al*, 1990).

Delagil, another synthetic antimalaria drug when administered parenterally had been found to inhibit blood coagulation and interfered with anticoagulant by forming complexes with heparin (Ovchinnikova *et al*, 1985).

2.3.6 MECHANISM OF ACTION OF CHLOROQUINE

Inside red blood cells, the malarial parasite must degrade hemoglobin to acquire essential amino acids, which the parasite requires constructing its own protein and for every metabolism digestion is carried out in a vacuole of the parasite cell.During this process, the parasite produces

the toxic and soluble molecule heme. The heme moiety consists of a porphyrin ring called Fe(II)-protoporphyrin IX (FP). To avoid destruction by this molecule, the parasite biocrystallizes heme to form hemozoin, a non-toxic molecule. Hemozoin collects in the digestive vaculole and insoluble crystals.

Chloroquine enters the red blood cell, inhabiting parasite cell, and digestive vacuole by simple diffusion. Chloroquine then becomes protonated (to CQ2+), as the digestive vacuole is known to be acidic (pH 4.7), chloroquine then cannot leave by diffusion. Chloroquine caps hemozoin molecules to prevent further biocrystallization of heme, thus leading to heme buildup. Chloroquine binds to heme (or FP) to form what is known as the FP- Chloroquine complex; this complex is highly toxic to the cell and disrupts membrane function. Action of the toxic FP-Chloroquine and FP results in cell lysis and ultimately parasite cell autodigestion. In essence, the parasite cell drowns in its own metabolic products (Hempelmann, 2007).

The effectiveness of chloroquine against the parasite has declined as resistant strains of the parasite evolved. They effectively neutralize the drug via a mechanism that drains chloroquine away from the disgestive vacuole. Chloroquine-resistant cells efflux chloroquine at 40 times the rate of vacuole, including sets of critical mutations in the gene (*Plasmodium falciparum*)-Chloroquine Resistance Transporter). The mutated protein, but not the wild-type transporter, transports chloroquine when expressed in *Xenopus* oocytes and is thought to mediate chloroquine leak from its site of action in the digestive vacuole (Martin *et al*, 2009).

2.3.7 CHLOROQUINE METABOLISM AND EXCRETION

When administered orally, chloroquine is rapidly and almost completely absorbed from the gastro intestinal tract with a bioavail-ability of 75-80% (Adelusi and Salako, 1982).

Other routes of chloroquine administration include subcutaneous, intramuscular and rectal. Maximum plasma concentrations are reached in 1-2hours (Ahmed *et al*, 2003) and remain up to 3.6hours after administration. Chloroquine has a large apparent volume of distribution in the body (160-800kg). It has a half-life ranging from 2-3days in rats and up to 3-6days in humans (Abdi *et al*, 1995). The distribution of chloroquine within human blood is also important because the malaria parasite is intraerythrocytic during schizogony. Chloroquine induced redistribution of a neutral aminopeptidase may be the cause of haemoglobin accumulation in endocytic vesicles of malaria parasites (Fitch *et al*, 2003). The liver transforms approximately 30-50% of the administered chloroquine, although extrahepatic sites of microsomal metabolism could also be of clinical significance in view of the extensive tissue distribution of chloroquine. Mono-desethylochloroquine is the main metabolite of chloroquine and it has been shown to have the same anti-malaria activity against chloroquine susceptible *Plasmodium falciparum* as the parent compound (Aderounmu, 1984).

2.4 LIFE CYCLE OF *PLASMODIUM BERGHEI* AND TRANSMISSION OF MALARIA PARASITE

Malaria infects a mosquito when the female takes a blood meal from an infected human or rodent. Along with the blood, the mosquito brings in male and female gametocytes cells. Only in the mosquito will the two forms combine to form a new plasmodium by sexual reproduction. When the two have combined, a diploid ookinette is formed that will penetrate the gut of the mosquito and take up residence in the abdomen. Once in the abdomen, the ookinette will form a cyst, within which will occur cell divisions producing a large number of asexual needle-like sporozoites. The sporozoites will work their way through the body of the mosquito towards the head and will settle in the salivary glands of the mosquito, ready to be injected back into its host. An infection in the rodent starts with the bite of an infected mosquito, which inoculates the

sporozoites in the blood stream of the host. The sporozoites are carried around the body until they pass into the liver.

In the liver, they penetrate liver cells and begin to transform into schizont. A mature schizont is a cell that ruptures, releasing many new cells called merozoites. Merozoites are the primary invasive cell of the life cycle in the human. Merozoites invade the liver cells, and also enter the blood stream where they invade red blood cells.

In the red blood cells, merozoites will become enlarged to form a ring-shaped cell with a single nucleus called ring trophoizoite. The nucleus of this cell will divide asexually and produce a multinucleated ring shaped cell, which will then begin to divide into more merozoites. The red blood cell ruptures, releasing merozoites and toxins into the blood stream.The toxins will cause the symptoms associated with malaria. The merozoites will find new red cells to invade immediately, where they will either repeat the pattern, or begin the second pattern of the life cycle.

In the second pattern, the ring trophoizoite never forms, instead, the merozoites metamorphoses into a male or female gametocyte. The gametocyte will travel in the blood waiting to be drawn into a feeding mosquito, where they will begin the mosquito portion of the life cycle.

2.4.1 HISTOPATHOLOGICAL CHANGES IN PLASMODIASIS

The most pronounced changes related to malaria involve the blood and the blood forming system; the liver and the spleen. In falciparum malaria, the hepatocytes may be involved causing functional changes as well (malaria hepatitis). According to the world health organization (WHO), jaundice is one of the cardinal manifestations of severe malaria. It results from either the intravascular haemolysis of parasitized erythrocytes or hepatic involvement.

Also, according to Masor in 1996, apart from jaundice, liver function disturbances were shown by an increase in bilirubin (Kochar *et al.*, 2003). Malaria infection caused massive liver injury with elevated alanine transaminase (ALT) and aspartate transaminase (AST) (Yoshimoto *et. al*, 1998). Changes in liver may result from alteration in blood flow through the organ as parasitized red blood cells adhere to endothelial cells, blocking the sinusoids and obstructing the intrahepatic blood flow, which is impairment of local microcirculation associated with hepatocellular damage. Plasmodium sporozoite invasion of liver cells was studied and migration damage to hepatocytes was confirmed with liver sections, revealing clusters of necrotic hepatocytes adjacent to structurally intact, sporozoites infected hepatocytes(Frevert *et al*, 2005).

Infections due to *P. falciparum* are by far the most dangerous. Victims of this "malignant tertian" form of the disease may deteriorate rapidly from mild symptoms to coma and death unless they are diagnosed and treated promptly and properly. The greater virulence of *P. falciparum* is associated with its tendency to infect a large proportion of the red blood cells. Patients infected with that species will exhibit ten times the number of parasites per cubic millimetre of blood than patients infected with the other three malaria species. In addition, red blood cells infected with *P. falciparum* have a special tendency to adhere to the walls of the tiniest blood vessels, or capillaries. This result in obstruction of the blood flow in various organs, but the consequences are gravest when capillaries in the brain are affected, as they often are. It is this latter complication—known as cerebral malaria and manifested by confusion, convulsions, and coma—that frequently kills victims of *P. falciparum* malaria (Encyclopædia Britannica, 2009).

Andréa *et al* (2008) stated thatsevere malarial anemia and cerebral malaria are the main complicationsof *Plasmodium falciparum* infection. They are responsible formost of the estimated

one to three million malaria-related deathsevery year in the world, mainly among children below 5 yearsof age in sub-Saharan Africa (Murphy and Breman, 2001). Severe malarial anemia isreported to be the earliest complication, usually affectingchildren below 2 years of age (Owusu-Agyei, *et al.*, 2002). Although severe anemia isa major concern in malaria pathology due to its high mortalityrates, milder forms of anemia also are important, since thismanifestation is responsible for considerable morbidity andis one of the major factors for the high disability-adjustedlife years attributed to malaria (Murray and Lopez, 1997). Iron deficiency, intestinal helminths, and human immunodeficiency virus infectionmake significant contributions to the pathogenesis of anemiain many African countries, but now there is substantial evidencesuggesting that malaria is indeed a major underlying factor(Muhangi, 2007).

Although it has been estimated, the real impact of malarialanemia on the affected populations is unknown. The few availabledata mostly are restricted to studies conducted in Africa (Nchinda, 1998).

Harry. (2006) in his work titled "Involvement of Gonadal Steroids and Gamma Interferon in Sex Differences in Response to Blood-Stage Malaria Infection" reported that males are more susceptible to many protozoan infections thanfemales and field and laboratory studies link increased susceptibilityto infection with circulating steroid hormones (Klein, 2004).One genus of protozoan parasites that causes a pronounced sexualdimorphism in vertebrate hosts is *Plasmodium*. Among humans, although the incidence of infection is often similar betweenthe sexes Weise (1979), sex differences in theintensity of infection are reported in which men have higherparasitemia than women. The observation that *P.falciparum* (i.e., a human malaria parasite) density increasesat puberty in men, but not in women, suggests that circulatingsex steroids may influence this outcome.

Studies of rodent malarias have confirmed that males are morelikely to die after blood-stage malaria infection than are females. Castration of male mice reduces, whereas exogenousadministration of testosterone increases, mortality after infectionwith *P. chabaudi* or *P. berghei* Wunderlich (2002). The immunosuppressiveeffects of testosterone may underlie increased susceptibilityto *Plasmodium* infections in males compared to females. Injectionof female mice with high doses of testosterone reduces antibodyproduction, the number of major histocompatibility complex classII cells in the spleen, and the expression of malaria-responsivegenes in the liver but does not affect cytokine production (Wildling, 1999).

2.4.2 HISTOMORPHOLOGIC CHANGES IN NAPHTHOQUINONE AND RELATEDCOMPOUNDS

Quinine

Quinine suppresses striated muscular tissue in two ways: directly by prolonging the refractory period after muscle contraction and indirectly by heightening the threshold at the neuromuscular junction. This is illustrated by the fact that small amounts of quinine increase the severity of myasthenia gravis symptoms. It was probably due to the observation that quinine reduces shivers, and that malaria is often accompanied by fever, that this was administered. It may have been pure chance that precisely this agent also killed the parasite and brought healing.

Quinine is a substance with highly irritating properties. Quinine may increase the secretion of insulin from the pancreas, with the risk of hypoglycaemia. Quinine allergy is not common. What is common is a range of side effects such as tinnitus, temporary deafness for high frequencies, headache, nausea and palpitations. These toxic phenomena are known as cinchonism. This reduces the patient's compliance. Quinine increases irritability of the pregnant uterus.

Chloroquine

Adelusi *et al,* (1982) reported that the use of chloroquine for the chemotherapy of malaria in Nigeria and parts of the tropics posed a problem of chloroquine accumulation in the kidney, liver and spleen, and its attendant toxic effect. Degeneration and regression of interstitial tissues and Leydig cells have been observed following treatment of rats with test doses of chloroquine. A decrease of androgen biosynthesis is therefore expected since these cells are the primary source of the male sex hormones. These results are in agreement with reports from other investigators who observed that chloroquine caused disruption of spermatogenesis due to insufficient production of androgens by Leydig cells, (Ebong *et al*, 1999). The regression observed in Sertoli and Leydig cells after treatment may suggest that chloroquine is toxic to the Sertoli and Leydig cells. Its toxicity is probably mediated via effects on the anterior pituitary. Okanlawon and Dyn (1996) had also reported that chloroquine exhibits anti-proteases activity and increase trans-epithelial resistance in immature Sertoli cells. Investigators had reported that toxicants that cause damage due to their irreversibility are those that kill or genetically alter spermatogonia or Sertoli cells, (Chaplin *et al*, 1984; Potashnik & Abeliovich, 1985; Mattison *et al,* 1990). Zukerman (1978) had suggested that dead sperm or immotile sperm often reflect the effects of post-testicular events and also reported that sperm count and sperm morphology have been found to provide indices for the integrity of spermatogenesis. The observed degeneration of germinal epithelium, interstitial tissues, Leydig cells, inter tubular stroma and loss of most of the spermatogenic cells in this study may probably lead to inhibition of spermatogenesis.

2.4.3 HISTOPATHOLOGIC CHANGES IN PLASMODIASIS IN VARIOUS ORGANS OF MALE AND FEMALE REPRODUCTIVE SYSTEM

Histological observation revealed well defined seminiferous tubules in the testis of control rats. Numerous enlongated Leydig cells when viewed individually were also present as well as Sertoli cells. The group treated with chloroquine phosphate, the seminiferous tubules was irregular, immature spermatozoa were seen lying in the lumen of seminiferous tubules accompanied by cell debris. In other groups seminiferous tubules appeared as isolates (Ahmad *et al,* 1989; Ashirce *et al,* 1991).

Russel *et al*,(1998) also reported that characteristic testicular lesion that is necrotic such as that of spermatogonia;spermatocytes and cap phase spermatids have been observed with several agents acting through different mechanisms. Such necrotic features were also observed in the study with chronic chloroquine treatment. The histopathology of the earlier testicular lesion found on day 7 of chloroquine treatment was oedema of the interstitial cells, Leydig cells and capillary dilation. Day 14 showed necrosis of seminiferous tubules cells oedema based tubule and disappearance of lumen with evidence of fibrosis. At day 21 there were the necroses of the seminiferous tubular cells, necrosis of the Sertoli cells and oedema of the basal lamina with the disapperance of lumen. Chloroquine therefore can acutely disturb the testosterone homestasis via the alteration of the interstitial cells or Leydig cells at the initial onset and chronically with the necrosis of seminiferous tubular cells and Sertoli cells, the low plasma testosterone level at day 7, 14 and 21 may therefore be as a result of the degeneration of Leydig cells which produce testosterone that is released into circulation unlike the testosterone produced from Sertoli cells which essentially is for spermatogenesis (Ishii-Ohba Maisumura *et al,* 1984).

It has also been reported (Singh and Chakravarty, 2000)that the testes of untreated controls and distilled water controls exhibited normal histological features; the seminiferous tubules showed spermatogenic activity with successive stages of transformation of

spermatogonia into spermatozoa. By contrast marked regressive changes were observed in the seminiferous tubules in testes of nitrofurazone-treated mice. In testes of mice treated with nitrofurazone for 10 days, there was marked depletion of germ cells in the seminiferous tubules and the germinal epithelium was lined mainly with Sertoli cells, spermatogonia, spermotocytes and a few wound spermatids. The tubules showed intraepithelial vacuoles and the occurrence of multinucleated giant cells contained 3-9nuclei of germ cells arranged either at the periphery or scattered in the cytoplasm of the cell body.

Giant cells formed with nuclei of early spermatid were more common than those with nuclei of spermatocytes. In testes of mice treated with nitrofurazone for 20 days, regressive changes in the tubules were further pronounced. In many tubules, there was severe depletion of germ cells and the epithelium consisted of a thin layer of sertoli cells and spermatogonia. The most advanced germ cells noticed in the tubules were pachytene spermatocytes and these cells presented a necrotic appearance. However, by 56 days after drug withdrawal, testes showed histological features similar to those seen in the controls (Singh and Chakravarty, 2000).

2.4.4 HISTOPATHOLOGIC CHANGES IN CHLOROQUINE AND RELATED COMPOUNDS

Adelusi *et al*, (1982) reviewed that the use of chloroquine for the chemotherapy of malaria in Nigeria and parts of the tropics poses a problem of chloroquine accumulation in the kidney, liver and spleen, and its attendant toxic effect. Degeneration and regression of interstitial tissues and Leydig cells have been observed following treatment of rats with test doses of chloroquine. A decrease of androgen biosynthesis is therefore expected since these cells are the primary source of the male sex hormones (Ebong *et al*, 1999). The regression observed in Sertoli and Leydig cells after treatment may suggest that chloroquine is toxic to the Sertoli and Leydig

cells. Its toxicity is probably mediated via effects on the anterior pituitary. Okanlawon and Dyn (1996) had also reported that chloroquine exhibits anti-proteases activity and increase trans-epithelial resistance in immature Sertoli cells. Investigators had reported that toxicants that cause damage due to their irreversibility are those that kill or genetically alter spermatogonia or Sertoli cells, (Chaplin *et al*, 1984; Potashnik and Abeliovich, 1985; Mattison *et al*, 1990). Zukerman(1978) had suggested that dead sperm or immotile sperm often reflect the effects of post-testicular events and also reported that sperm count and sperm morphology have been found to provide indices for the integrity of spermatogenesis. The observed degeneration of germinal epithelium, interstitial tissues, Leydig cells, inter tubular stroma and loss of most of the spermatogenic cells in this study may probably lead to inhibition of spermatogenesis.

Nitrofurazone treatment in mice caused regressive histological changes in the seminiferous tubules resulting in the suppression of spermatogenesis. Furthermore, the antispermatogenic effects induced by nitrofurazone in testes of mice were reversible; this is consistent with the findings of Nelson and Steinberger (1952) and Paul *et al*(1953) in the rat after treatment with nitrofurazone. Thesestudies showed that the most advanced germ cells noticed in the regressed seminiferous tubules were pachytene spermatocytes, though a few round spermatids were sometimes observed in the tubules in testes of mice treated with the drug for 10 days. Nelson and Steinberger (1952) and Paul *et al* (1953) have also reported the inhibition of spermatogenesis at the pachytene spermatocyte stage in rate testes following nitrofurazone treatment. Multinucleated gaint cells as observed in the seminiferous tubules in testes of drug-treated mice are also reported in rat testis after nitrofurazone treatment (Miyaji *et al,* 1964). It is pertinent to note that such giant cells have also been reported in mouse testes after efferent duct ligation, vascectomy, (Singh and Chakravarty 2000) and treatment with several

antispermatogenic agents; in these experimental conditions, the testis exhibited regressive changes in the seminiferous tubules. The occurrence of giant cells in the testis is considered to be an expression of germ cell degeneration (Singh *et al*, 1999).

Hagenas *et al*, (1978) also reported that nitrofurazone acts directly on germ cells and thereby causes arrest of spermatogenesis. In their study, intraepithelial vacuoles were noticed in the seminiferous tubules in testes of nitrofurazone-treated mice. It is pertiment to mention here that Hoffer, (1983) has also reported occurrence of intraepithelial vacuoles in affected seminiferous tubules in rat testes after gossypol treatment, and that these vacuoles occurred primarily in the Sertoli cells. Such vacuoles are also reported to occur in the Sertoli cells after several kinds of testicular injuries, and they have, however, often been interpreted as a nonspecific reaction of these cells (Fawcett, 1975). Thus, it is difficult to say in mice as to how nitrofurazone treatment induces antispermatogenic effect in the testes, though the possibility of a direct action of the drug on the germ cells can not be ruled out.

Previous studies have also reported antifertility effects of some antimalarial agents such as quinine, artemisinin, halofantrine and chloroquine (Nwanjo *et al*, 2007). The most common abnormality in morphology observed for artemisinin was curved mid-piece which is also a secondary and tertiary aberration, but this was associated with high dose and long duration of use at recommended dose (Nwanjo *et al*, 2007). Furthermore, halofantrine have been reported to induce formation of immature spermatocytes which would result in the reduction of viable sperms (Didia *et al*, 2002). However, ciprofloxacin have been reported not to have any effect on the sperm morphology of healthy male Wistar rats,(Marefat *et al*, 2008) although, recognizable histological damage associated with a mild decrease in testicular volume and sperm concentration on healthy rats have been reported (Adel *et al*, 2000). There has not been any

report on the effect of the combination of ciprofloxacin and chloroquine on sperm motility and morphology.

The combination ciprofloxacin and chloroquine at the concentrations used in this study gave an increase in the percentage aberration when compared with the pure chloroquine but the values obtained are similar to those obtained at the two concentrations of ciprofloxacin used in this study. The percentage aberration obtained for groups 1 and 2, i.e. combination of 12.5mg chloroquine with 5 and 10mg/kg of ciprofloxacin were not significantly from that obtained for pure ciprofloxacin at 5 and 10mg/kg respectively. This suggests that whatever effect the combination of the two drugs may have on the percentage aberration does not differ significantly different from that obtained with the pure ciprofloxacin at the two concentrations used in the study.

2.5 THE LIVER

The liver is the largest organ in the body of mammals. It is the most active and most complex organ. It lies in the upper right quadrant of the abdomen beneath the diaphragm. It is protected by the ribs and held in place by ligamentous attachments. It has four lobes, the right one being the largest while the left one is smaller and wedged shaped. The last two lobes are very small and lie on the undersurface of the liver close to a pear shaped sac, the gall bladder, which acts as a reservoir for bile before it is discharged into the duodenum. The portal fissure is the name given to a cleft on the undersurface of the liver where various structures enter and leave the organ.

Entering the liver is the portal vein carrying blood from the stomach, spleen, pancreas and intestines and the hepatic artery carries arterial blood to the liver. The hepatic vein leaves the liver to carry blood to the inferior vena cava the right and left hepatic ducts carry bile to the gall

bladder. The liver is divided into many small circular units known as liver lobules. Each liver lobule is made of rows of cells, the hapatocytes, radiating from the central vein (Ochei and Kolhatkar, 2000).

2.5.1 FUNCTIONS OF THE LIVER

The liver plays a major role in metabolism and has a number of functions in the body which include:

i. **Glycogen Storage**

This occurs during carbohydrate metabolism, where glucose is converted to glycogen and stored in the liver. When glucose is required in the body, maybe during fasting, the glycogen is re-converted to glucose (glycogenolysis). Thus, blood glucose level is maintained but if the stored glycogen has been exhausted by the liver, and the blood glucose level is low, the liver can then convert proteins and fats into glucose (gluconeogenesis).

ii. Synthesis of cholesterol, phospholipids endogeneous triglycerides and lipoprotein occurs mainly but not exclusively in the liver. Some proteins such as albumin, plasma proteins are synthesized in the liver. Many of the coagulation factors, fibrinogen, prothrombin, factor V, VII, IX, X, XI and XII are manufactured in the liver (Baker and Silverton, 2001).

iii. **Excretory Functions**

The liver plays a role in the excretion of substances from the body, example of such substances include cholesterol, which is excreted in the bile either unchanged or after conversion to bile acids, amino acids, which are deaminated in the liver, steroid hormones, which are metabolized and inactivated by conjugation with gluconate and

sulphate, and excreted in urine. Many drugs are metabolized and inactivated by enzymes of the endoplasmic reticulum system; some are excreted in the bile.

iv. **Protection and Detoxification**

The liver also helps in the detoxification of toxic substances that have been absorbed from the gastro intestinal tract (Crook, 2006). The Kupffer cells which forms a part of mononuclear phagocytic defence system (a part of the reticuloendothelial system) are involved in the normal destruction of erythrocytes, and also the removal of micro-organisms.

v. **Circulatory Function**

The largest blood reserve is in the liver. It is in the regulation of blood volume in the body. The liver plays a role in immunologic defence through its reticuloendothelial system, helps to regulate blood volume by serving as a blood storage area, and is a means for mixing the blood from the partial system with that of systemic circulation.

2.5.2 THE LIVER ENZYMES (ASPARTATE TRANSAMINASE, ALANINE TRANSAMINASE)

Amino transferases are a group of enzymes that catalyse the transfer of an amino group from alpha-amino acid to alpha oxo-acids. This is an important step in the metabolism of amino acids. All naturally occurring alpha-amino acids can take part in such reactions, different enzymes are being involved but two are clinically important. They are:

a. L-Aspartate amino transferase AST or formally GOT (Glutamic oxoglutarate amino transferase).

b. L-Alanine amino transferase ALT or formally GPT (Glutamic pyruvic amino transferase).

The detection of high levels of these liver enzymes suggests liver damage. Under normal circumstances, these enzymes reside within the cells of the liver. But when the liver is injured, these enzymes are spilled into the blood stream.

Among the most sensitive and widely used of these liver enzymes are the amino transferases. Elevated levels are quite sensitive for liver injury meaning that they are likely to be present if there is liver injury. However, they may also be elevated in other conditions such as viral hepatitis, bacterial infection, heart failure, drugs etc.

In general, any damage to the liver will cause medium elevation in these liver enzymes (amino transferase). However, very high elevations of the transaminases suggest severe liver damage such as viral hepatitis, liver injury from lack of blood flow, or injury from drugs or toxin. Most disease processes cause alanine transaminase to rise higher than aspartate transminase, example is alcoholic hepatitis due to excessive intake of alcohol. Alanine transaminase levels double or triple that of aspartate transminase are consistent in alcoholic liver disease. Levels over one thousand (1000) can be associated with ischemic hepatitis. Mildly high values are seen in cases of severe bacterial infections, malaria infection, liver cirrhosis, and pericarditis.

2.6 RED BLOOD CELLS (Erythrocytes)

The first person to describe red blood cells was the young Dutch biologist Jan Swammerdam, who had used an early microscope in 1658 to study the blood of a frog. Unaware of this work Anton Van Leeuwenhoek provided another microscopic description (Red Gold-Blood History, 2007).

The red blood cells are the most common type of blood cells and vertebrate's body's principal means of delivering oxygen to the body tissues via the blood. They take up oxygen in

the lungs or gills and release it while squeezing through the body's capillaries. They consist mainly of hemoglobin, a biomolecule that can bind to oxygen and the oxygen can diffuse easily through the cell membrane of the red blood cell. The size of erythrocytes varies widely among vertebrate species. Erythrocyte width is on average about 25% larger than the diameter of the capillary and it has been hypothesized that this improves the oxygen transfer from erythrocytes to tissues (Snyder *et al*, 1999). The only known vertebrate without erythrocytes are the crocodile, icefishes from family *Channichthyidae*; this is because they live in very oxygen rich cold water and transport oxygen freely dissolved in their blood. They don't use heamoglobin anymore; remnants of heamoglobin genes can be found in their genome (Ruud, 1954).

Erythrocytes in mammals have nuclei in the early phases of development but are anucleate when they mature, meaning they lack a cell nucleus in order to provide more space for heamoglobin. Erythrocytes of other vertebrates have nuclei with the exceptions of *Salamanders* of the *Batrachoseps* genus and fish of the *Maurolicus genus* with closely related species (Wingstrand, 1956).

Apart from the function of the red blood cell in oxygen transportation, erythrocytes play a part in body's immune response; when they are lysed by pathogens such as bacteria, their haemoglobin release free radicals that break down the pathogen's cell wall and membrane thereby, killing it. They also release ATP which causes the vessel walls to relax and dilate when they undergo shear stress in constricted vessels.

Erythrocyte disk of a typical human has a diameter of 6-8µm and a thickness of 2µm. A normal erythrocyte has a volume of about 90fl and about a third of this volume is heamoglobin. Adult humans have roughly $2\text{-}3x10^{13}$ red blood cells at any given time with women having about

4 to 5 million erythrocytes per microliter (cubic millimeter) of blood and men with about 5 to 6 million. Red blood cells are thus much more common than other blood particles.

The process of producing red blood cells is called erythropoiesis and red blood cells are continuously produced in the red bone marrow of large bones at a rate of about 2 million per second. Before and after leaving the bone marrow, the developing cells are known as reticulocytes. Erythrocytes develop through reticulocyte to mature erythrocytes in about 7 days and live for about 100-120 days. The aging erythrocytes will undergo changes in its plasma membrane making it susceptible to be recognised by phagocytes and subsequent phagocytosis in the spleen, liver and bone marrow. Almost all aged erythrocytes are removed from the circulation before they are old enough to hemolyze (Dean, 1999).

2.7 White blood Cells (Leucocytes)

Blood is a liquid tissue. When the red blood cells are removed from blood, a watery plasma or serum remains and white blood cells are suspended in this liquid. White blood cells (WBC) are the key players of the immune system and they defend the body against infectious organisms and foreign substances. White blood cells are produced in the bone marrow. All white blood cells arise from haematopoietic stem cells and these stem cells are very rare, representing only one of every 10,000 bone marrow cells. The cell division is by a process of mitosis, forming either more stem cells or white blood cells that can differentiate into specific white cell types such as the lymphocytes.

The white blood cell count is a measurement of the number of WBCs present in one milliliter of blood. The cells proliferate, increasing dramatically in response to bacterial infection. An increased count (leukocytosis) can be seen in infection, stress and in various blood disorders and malignancies including Leukaemia. The early immature cells like band,

metamyelocytes, mylocytes, promyelocytes and blast cells are not normally seen in the blood but are seen in some conditions particularly, when white blood cells production increases dramatially (Moore, 2002). The whtie blood cells count represents the basic types of white blood cells in percentages. About 50-60% of the white blood cells are neutrophils, 05-2.0% are eosinophils and basophils, 20-40% is lymphocytes and 2-9% is monocytes (Moore**,** 2002).

2.8 Packed Cell Volume(Hematocrit)

The hematocrit is the percentage of blood that is comprised of red blood cells. A small volume of blood drawn from the finger-tip could be used for heamtocrit testing. A capillary tube is often used for this test and it could also be done using an automated instrument as part of a complete blood count. The purpose of this test is to measure or determine the extent of aneamia in an individual. Conditions such as vitamin B12 deficiency, pregnancy, folic acid deficiency, malnutrition, liver and kidney diseases, chronic or acute blood loss, iron deficiency etc could result in decreased hematocrit while an increased hematocrit is associated with shock, severe burns, diarrhea, and polycythemia.

The packedcell volume (PCV) can be determined by filling a capillary tube with the blood and the other end of the tube sealed and spun or centrifuged at 3,000 RPM for 5 minutes. This separates the blood into layers as the tube spins. The denser blood cells which are the red blood cells settle at the bottom of the tube, the buffy coat which consists of the white blood cell and the liquid plasma which rises to the top. The height of the red cell column is measured as the PCV in percentage (Purves *et al*, 2004).

The normal values vary with age and sex. Some changes are:

At birth: 42-60%

6-12 months: 33-40%

Adult males: 42-52%

Adult females: 35-47% (Kjeldsberg, 2000).

These parameters; PCV, WBC and RBC counts could be affected by many medications and some drugs could be toxic to them thereby rendering them dysfunctional. So, it is imperative to study these cells counts in both health and disease states.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Animals

Two hundred male albino Swiss strain mice (18-22 g) obtained from from the National Veterinary Research Institute (NVRI) farms, Vom, Plateau State, Nigeria were used. All the mice were quarantined and aclamatized for four weeks before the commencement of the study. The mice were all fed with pelletized mice grower and water*ad libitum.* The animals were randomly allotted to experimental groups as shown in table 1 for naphthoquione which was similarly carried out for chloroquine as the standard drug used in this study.

Table 1: Experimental grouping

Treatment groups (N = 10 in each group)
Grp I-Control (0 mg/kg)
Grp II- 0.1mg/kg naphthoquinone
Grp III- 0.5mg/kg naphthoquinone
Grp IV-1mg/kg naphthoquinone
Grp V -2mg/kg naphthoquinone
Grp VI-*Plasmodium berghei* infected mice
Grp VII-*Plasmodium berghei* infected + naphthoquinone (0.1mg/kg) mice
Grp VIII-*Plasmodium berghei* infected naphthoquinone (0.5mg/kg)mice
Grp IX-*Plasmodium berghei* infected naphthoquinone (1mg/kg) mice
Grp X-*Plasmodium berghei* infected naphthoquinone (2mg/kg) mice

3.2 ADMINISTRATION OF THE DRUGS

The drug naphthoquinone was weighed and dissolved in Tween 20. Different concentrations of the drugs were administered to the 9 test groups of experimental mice. The drug was administered intraperitioneally with the use of a 2ml syringe and needle for 7 days. Control mice received water or Tween 20 as appropriate.

3.3 INFECTION OF MICE WITH *PLASMODIUM BERGHEI* PARASITE

Anka 1 N-strain of the malaria parasite, *P. berghei*, maintained in mice by serial passaging from mouse to mouse was used throughout the experiment. The *Plasmodiumberghei* infected donor micewere obtained from the Nigerian Institute of Medical Research, Lagos, Nigeria. Each mouse was subsequently given standard intra-peritoneal inoculums of *P.berghei* parasites with the aid of a 1ml disposable syringe.The method of blood-induced *Plasmodium* infection in mice first described by Peters (1948, 1967) and used by others (Makinde and Obih, 1985) was employed in the present study.

3. 4 DETERMINATION OF POTENCY AND PARASITEMIA

Blood samples were collected from the tails of infected mice and thin blood smears were prepared, fixed with methanol, and stainedwith a 1:10 dilution of Giemsa stain 1x phosphate buffer (pH 7.1). Parasites werevisualized under a 100x oil immersion microscope, and parasitemiawas calculated by counting the number of parasites/total numberof erythrocytes in a minimum of three random fields. Significant parasitemia was observed.

3.5 Autopsy

At the end of each experimental period, mice were killed by cervical dislocation. Blood was collected immediately by cadiac puncture. Microscope slides were smeared with a thin film of the blood and the percentage parasitemia level was determined.

3.6 COLLECTION OF BLOOD SAMPLE

Each mouse in a group was put in a desicator and a cotton wool soaked with chloroform was placed inside. The mouse was made to inhale the chloroform which in turn knocks off the mouse. The mouse was brought out and placed on a Wooden Bench. A clamp was used to clamp the hands and legs of the mouse. A pair of scissors was used to cut open the mouse. The blood sample was collected through cardiac puncture with the use of syringes and needle and transferred into a z_{10} bottle. The blood was allowed to clot and afterwards centrifuged using 3000rpm for 5 minutes. The serum was transferred into a plain bottle and refrigerated at 20^{o}C until the day for the analysis.

3.7 DETERMINATION OF SPERM MOTILITY, VIABILITY AND COUNTS

Collection of Epididymal Fluid

The mice were anaesthesized using chloroform 3 days after the administration of the drugs. The cauda epididymis was dissected out immediately and the epididymal volume was determined(approximately 0.01ml per mouse). The testes were carefully exposed and one of them was removed together with its epididymis. The epididymis was separated and the epididymal fluid was collected from the caudal part and the progressive sperm motility, sperm count, live/dead ratio (viability) and sperm morphology were determined as described earlier (WHO, 1987, Farag *et al*, 2000).

Sperm motility:

Progressive sperm motility evaluation was done immediately after semen collection. Two drops of semen were placed on a microscope slide and two drops of warm 2.9% sodium citrate were added. The slide was then covered with a cover slip and examined under the microscope using 40×objectives with reduced light.

Sperm viability:

Sperm viability was done using the eosin/nigrosin stain. The dead sperm took up the stain. Sperm morphology was carried out by means of the Walls and Ewas stain as described by Raji *et al*., (2006).

Sperm Counts:

Epididydmal sperm count was done by homogenizing the epididymis in 0.5ml of Normal saline and counts were done by using the haemocytometer method (i.e using the improved Neubauer counting chamber). The central large square with 25 smaller squares was used. The 25 smaller squares within the central large square give a total area of $0.2mm^3$. The counting chamber was covered with a clean coverslip and charged by allowing the diluted sample to pass into the chamber using a Pasteur pipette by capillary action. The volume contained in the chamber is 0.1mm. The cells were allowed to settle for 3 minutes before counting.

5 squares of the 25 were counted and total number of cells was calculated as follows:

Total number of cells = N x 1/area(mm) x 1/Depth x DF, where N = number of cells counted

Area = 0.2mm, Depth = 0.1

DF = Dilution factor = 0.01 in 0.5

= 0.5/0.01=50

This calculation method was adopted for all the groups (i.e control and treatment).

3.8 MEASUREMENT OF TESTOSTERONE

The microwell testosterone EIA is a solid phase enzyme immunoassay utilizing the competitive binding principles. Testosterone present in the sample will compete with enzyme labeled testosterone for binding with anti- testosterone antibody immobilized on the microwell surface. The amount of conjugate that binds to the microwell surface will decrease in proportion to the concentration of testosterone in patient sample.

The unbound sample and conjugate are then removed by washing and the colour development reagents (substrates) are added. Upon exposure to the bound enzyme, a colour change will take place. The intensity of the colour reflects the amount testosterone conjugate and is inversely proportional to the concentration of testosterone in the sample within dynamic range of the assay. After stopping the reaction, the resulting colour is measured using a spectrophotometer at 450nm. The testosterone concentration in the sample and concurrently run controls can be determined from the standard curve.

PROCEDURE

A. PREPARATION

1. The unopened zip-lock microwell bag, test components and sample specimens were brought to room temperature prior to testing.
2. A working dilution of washing buffer from the 10x concentrate provided was prepared and 30ml of washing buffer 10x with 270ml of distilled water were mixed.
3. Samples were diluted with expected testosterone concentration over 20ng/ml with Dilution Buffer mixed thoroughly before assaying.

4. The number of coated microwells needed was determined and a data sheet was marked with the appropriate information. Standard and controls required at least one well each. However the test was performed in duplicate to ensure accuracy.

INCUBATION

1. The desired number of microwells was removed from the zip-lock bag and placed in a well holder
2. 25µl of each reference standard, control and test sample was dispensed into the appropriate well within 5 minutes.
3. 50µl of enzyme conjugate was dispensed into each well
4. The wells were gently rocked for twenty seconds then sealed (by covering) with parafilm or other film sealant.
5. They were then incubated at room temperature for 60 minutes
6. Cover seal was removed and discarded. The incubation mixture was decanted thoroughly by flicking into a sink with disinfectant.
7. The microwells were then washed three times with diluted washing buffer
8. The wells were dried by firmly rapping the plate on a clean paper towel to remove excess washing solution.

COLOUR DEVELOPMENT

1. One drop of substrate reagent A and one drop of substrate reagent B was dispensed into each well. The wells were gently rocked for twenty seconds and incubated at room temperature (15^{o} – 28^{o}C) for 15 minutes
2. The reaction was stopped by adding one drop of stopping solution to each well and gently rocked the wells.

3. The absorbance was read at 450nm (A_{450}) of each within 30 minutes after stopping the reaction.

3.9 MEASUREMENT OF LUTEINIZING HORMONE (LH)

The Microwell luteinizing EIA is a solid-phase enzyme immunoassay based on the 'sandwish' principle. Two separate antibodies directed against distinct antigenic determinants of the LH molecules are utilized in the assay. The LH present in test sample reacts simultaneously with one antibody immobilized on the microwell surface and with another antibody conjugated to horse-adish peroxidase enzyme. So an antibody – antigen – antibody emzyme complex is formed on the microwell surface.

Then the unbound components are removed by washing and the colour development reagents (substrates) are added. Upon exposure to the enzyme, a colour reflects the amount of bound anti-LH enzyme conjugate and is proportional to the concentration of LH in the specimen within the dynamic range of the assay. After stopping the reaction the resulting colour is measured at 450nm using a spectrophotometer. Using a standard curve obtained by plotting the Leitinizing hormone concentrations of reference standards versus the corresponding absorbance, the Latinizing hormones concentrations of the concurrently run tests samples and controls can now be determined.

PROCEDURE

1. The unopened zip-lock microwell bag, test components and sample specimens were brought to room temperature prior to testing.
2. A working dilution of washing buffer was prepared from the 10x concentrate provided. Mix 30ml of washing buffer 10 with 270ml of distilled water.

3. Determine the number of coated microwells needed was determined and a data sheet was marked with the appropriate information with an extra well included (designated A) for substrate blank. The standards and controls were run in duplicate to ensure accuracy

B. INCUBATION

1. The desired number of microwells were removed from the zip-lock bag and placed in a well holder
2. 50µl of each reference standard, control and test sample was dispensed into the appropriate well (excluding A1) within 5 minutes
3. One drop of enzyme conjugate was dispensed into each well
4. The wells were gently rocked for twenty seconds then sealed with parafilm or other film sealant.
5. They were then incubated at 37^{o}C for 30 minutes
6. Cover seal was removed and discarded. The incubation mixture was discarded thoroughly by flicking into a sink containing disinfectant.
7. The microwells were washed five times with diluted washing buffer.
8. The wells were dried by firmly tapping the plate on a clean paper towel to remove excess washing solution.

COLOUR DEVELOPMENT

1. One drop of substrate reagent A and one drop of substrate reagent B was dispensedinto each well (including A1) and gently rocked for twenty seconds. The well was then incubated at room temperature (15^{o} - 28^{o}C) for 10 minutes.
2. The reaction was stopped by adding one drop of stopping solution to each well and gently rocked.
3. The absorbance of each well was read at 450nm (A_{450}) against the substrate Blank (well A1) within 30 minutes after stopping the reaction.

3.10 MEASUREMENTOF FOLLICLE STIMULATING (FSH)

The Microwell FSH EIA is a solid-phase enzyme immunoassay based on the 'sandwich' principles Tween separate antibodies directed against district antigenic determinate of follicle stimulating hormone molecule are utilized in the assay. The follicle stimulating hormone present in the test sample reacts simultaneously with one antibody immobilized on the microwell surface and with another antibody conjugated to horseradish peroxidase enzyme. So an antibody conjugated to horseradish peroxidase enzyme. So an antibody – antigen – antibody – enzyme complex is formed on the microwell surface.

Then the unbound conjugate is removed by washing and the colour development reagent (substrates) is added. Upon exposure to the enzyme, a colour change well take place. Intensity of the colour reflects the amount of bound anti follicle stimulating hormone enzyme conjugate and is proportional to the concentration of follicle stimulating hormone in the specimen within the dynamic range of the assay. After stopping the reaction the resulting colour is measured using a spectrophotometer at 450nm. Using a standard curve obtained by plotting the follicle stimulating hormone concentrating of reference standards versus the corresponding absorbance, the follicle

stimulating hormone concentrations of the concurrently run test sample and controls can now be determined. The procedure was similar to that of LH except that specific FSH kit was used.

3.11 HISTOLOGICAL PROCEDURES

The mice were placed in a dessicator containing chloroform and were anestisized. The testes were dissected out and weighed. The selected tissue was sliced, placed in a tissue cassette and properly labeled. The testes were then fixed for histological studies in freshly prepared 10 % formal saline, dehydrated in a descending graded alcohol, cleared in xylene, infiltrated and embedded in paraffin wax. Tissues were sectioned at 6µ, and the sections were stained with Periodic Acid-Schiff (PAS) method and the Harris haematoxylin. The tissue was also stained with Haematoxylin and Eosin (H&E) method (Drury *et al*, 1976). The slides were examined microscopically with low power objective (X10) and photo micrographs were taken.

3.12 ASPARTATE TRANSAMINASE ANALYSIS

PRINCIPLE

Oxaloacetate reacts with aspartate transaminase which decarboxylates it spontaneously to pyruvate which is measured by hydrazone formation (after the pyruvate has reacted with 2,4, dinitrophenyl hydrazine).

METHOD OF ANALYSIS

Aspartate transaminase was determined by an autoanalyser, (Hitachi 902).

3.13 ALANINE TRANSAMINASE ANALYSIS

PRINCIPLE

The pyruvate produced by the transamination activities of alanine transaminase reacts with 2, 4 dintrophenyl hydrazine to hydrazone.

METHOD OF ANALYSIS

Alanine aminotransferase was determined by an autoanalyzer, (Hitachi 902).

Determination of superoxide dismutase

Superoxide dismutase (SOD) activity was evaluated using the RANSOD Kit (Randox, Crumlin, England), with one modification. Instead of whole blood samples, homogenized and filtered testis solution was used. The kit contained mixed substrate (xanthine, 0.05 mmol/L and I.N.T. 0.05 mml/L), buffer (CAPS 40.00 mmol/L, pH 10.2, EDTA, 0.94 mmol/L), xanthine oxidase standard (80 U/L), sample diluents (5.40 U/L) and phosphate buffer (0.01 mol/L, pH 7.0) (50.00 mL of 0.20 mol KH2PO4 + 29.65 mL of 0.20 N NaOH made up to 1 L, with distilled water). The percentage inhibition of each sample was used to obtain the SOD units from a standard curve of the reconstituted and diluted RANSOD kit.

Assessment of Lipid Peroxidation (LPO)

Lipid peroxidation was assayed by measuring the thiobarbituric acid reactive products (TBARS) present in the test sample. This was based on the method of Vashney and Kale (1970) and expressed as micromolar of malondialdehyde (MDA/g tissue). It is based on the principle that the ratio of chromogenic reagent (2-thiobarbituric acid) to MDA (an end point of LPO) under acidic condition to yield a stable pink chromophore read with a spectrophotometer at a maximum absorption of 532nm wavelength. Malondialdehyde level (units/mg protein) was calculated according to the method of Adam-Vizi and Sergei (1987) with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}cm^{-1}$.

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance} \times \text{Volume of mixture}}{E_{532nm} \times \text{Sample volume} \times \text{mg protein}}$$

Catalase Assay

Catalase activity was determined according to the method of Sinha (1971). This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an intermediate. The chromic acetate then produced is measured calorimetrically at 570-610 nm wavelength. Since dichromate has no absorbance in this region, the presence of the compound in the assay mixture does not interfere with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction is stopped at a particular time by the addition of dichromate/acetatic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate calorimetrically after heating the reaction mixture.

Reagents:

1. 5% Potassium dichromate solution (K_2CrO_7). 1.25g of dichromate was dissolved in 200ml of distilled water and made up to 25ml.
2. 0.2M Hydrogen peroxide. 1.150ml of 30% (w/v) H_2O_2 was diluted with distilled water in a volumetric flask and the solution made up to 50ml.
3. 0.1M Phosphate buffer (pH 7.0). 0.496g of di-potassium hydrogen orthophosphate, K_2HPO_4 and 0.973g of potassium di-hydrogen orthophosphate, KH_2PO_4, wre dissolved in 90ml of distilled water. The pH was adjusted to 7.0 and then made up to 100ml distilled water.
4. Dichromate/Acetic acid solution. The reagent was prepared by mixing one part of 5% solution K_2CrO_7 with three parts of glacial acetic acid i.e ratio 1:3 (75ml).

3.14 STATISTICAL ANALYSIS

Data were expressed as mean ± SEM and analysed using Student's *t*test and ANOVA where necessary and $p<0.05$ was considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 CHLOROQUINE STUDIES

In the chloroquine treated mice, the percentages of sperm motility and viability and the number of sperm cells ($x10^6$/ml) significantly reduced ($p<0.05$) with all concentrations tested when compared with their control counterparts. *Plasmodium berghei* infection similarly reduced the sperm indices. Chloroquine seems to produce no significant effect in the sperm indices in *Plasmodium berghei* infected mice (table 2).

Table 2: Effect of chloroquine on sperm indices in mice

Treatment groups	Sperm viability (%)	Sperm motility (%)	Sperm counts (million/ml)
Group I-Control 0 mg/kg	87.33 ± 4.04	72.33 ± 2.08	21.17 ± 2.62
Group II-5mg/kg chloroquine	81.00 ± 1.73*	62.33 ± 3.21*	13.33 ± 1.38
Group III-10mg/kg chloroquine	76.33 ± 1.52*	59.00 ± 3.61*	12.33 ± 0.58*
Group IV-15mg/kg chloroquine	69.33 ± 1.15*	50.00 ± 2.00*	11.33 ± 1.26*
Group V-20mg/kg chloroquine	64.33 ± 4.04*	42.00 ± 2.00*	9.75 ± 0.90*
Group VI-*Plasmodium berghei* infected	84.23± 3.02	53.03 ± 3.12*	20.07 ± 2.32
Group VII-*Plasmodium berghei*infected+ chloroqiune (5 mg/kg) treated	70.03 ± 3.20*	52.02 ± 2.02*	19.09 ± 1.82*
Group VIII-*Plasmodium berghei* infected chloroqiune (10 mg/kg) treated	70.00 ± 2.50*	51.10 ± 2.10	20.00 ± 1.68
Group IX-*Plasmodium berghei* infected chloroqiune (15 mg/kg) treated	69.25 ± 2.61*	50.15 ± 2.11	20.02 ± 2.10
Group X-*Plasmodium berghei* infected chloroqiune (20 mg/kg) treated	65.01± 2.06*	48.24 ± 2.03	19.01 ± 1.27

*p< 0.05
Values are expressed as mean ± SEM
n = 10

4.1.1 Effect of chloroquine on haematological indices in male mice

Table 2 and figure 1 show the distribution of means and standard deviations of haematological indices in the control and chloroquine treated mice. There was a decreased in the number of RBC with an increase in concentration of the drug (as shown in table 3).

The WBC count also showed the same pattern with that of RBC, where the mean values were decreased with increase in concentration of the drug. Group II had the highest mean value of 6.51±3.99 compared to control that had a mean of 3.90 ±1.72. This showed a significant difference between group II (5mg) and group I (control) (6.51 and 3.9). In granulocytes, the test groups mean values were shown to be higher than the control 28.0 ±3.77 as shown in table 2 and figure 1. Group II had the highest mean value of 52.75 ±6.91, followed by group V 45.85 ±7.13. Group III was shown to have the highest mean value of 49.85±3.42 in Lymphocytes among the test groups when compared to the control that had a mean of 59.08 ±5.51. There was a fall in group II 34.68 ±7.24 which then increased in group III 49.85 ±3.42 and then starts to decrease with concencetration.

Also, in monocytes, an undefinite pattern was shown (table 3) along the groups. The mean values of the test groups were higher than the control. Group III had the highest mean of 15.98 ±2.05 and the control 10.75 ±1.04. The platelets mean values were shown to decrease with increase in the concentration of the drug even though a fall in the value was observed in group II 300.75 ±136.43 compared to the control group 370.0 ±145.90. Group v had the lowest mean of 44.0±2.30. A decreased platelet number was observed in the test groups and a very sharp decrease in group V. The Hb and PCV values were shown to decrease as the concentration is increased, but a higher value were observed 8.28 ±510.84 and 30.90 ±5.36 in group II compared to control 7.75 ±2.14. Red cell indices (MCV, MCH and MCHC) mean values did not follow

definite pattern within the groups. Group III had the higher mean value in MCV and MCH, 52.25±2.22 and 13.50±0.42 respectively and the lowest in group IV 44.13, ±2.32 and group II 11.98 ±2.52 respectively. In MCHC, the mean value was highest in group IV 27.67 ±1.68 compared to control 27.10 ±1.2. Chloroquine did not produce significant effect on the cells morphology but had on the cells count.

Table 3: Effect of chloroquine on haematological indices in *Plasmodium berghei* infected male mice

Groups	RBC (million /mm^3)	WBC	GR%	Lymp	Mono %	Plat	Hb (g/dl)	PCV(L/L)	MCV (FL)	MCH (Pg)	MCHC (g/dl)
1 Control	5.71± 1.17	3.90± 1.72	28.00± 3.77	59.08± 5.51	10.75 ± 1.04	370.00 ± 145.90	7.75± 2.14	28.43± 7.32	48.65± 4.81	12.45± 0.53	27.10± 1.21
II	6.70± .24	6.51± 3.99	52.75± 6.91	34.68± 7.24	13.50 ± .75	300.7± 136.43	8.28± 1.84	30.90± 5.36	46.03± 7.87	11.98± 2.52	26.58± .95
III 10mg	5.50± .27	3.83± 1.09	32.78± 4.90	49.85± 3.42	15.98 ± 2.05	352.75 ± 88.82	7.40± 0.49	28.70± 1.92	52.25± 2.22	13.50± 0.42	26.80± 1.15
IV 15mg	3.69± .36	2.94± 0.60	35.33± 18.92	44.73± 13.29	10.90 ± 3.02	259.50 ± 26.10	4.58± 0.90	16.38± 2.12	44.13± 2.32	12.35± 0.96	27.67± 1.68
Grp V 20mg	1.76± .26	1.66± 0.88	45.85± 7.13	37.03± 3.82	12.70 ± .91	404.00 ± 2.30	2.13± 0.25	8.45± 0.94	48.30± 2.68	12.48± 0.96	25.03± 1.17
VI- P.B	5.40 ± 0.41	1.56± 0.88	43.85± 7.53	34.78± 7.54	16.98 ± 2.07	302.72 ± 134.13	6.30± 0.40	13.28± 2.02	47.05± 7.55	12.78± 2.42	25.83± 2.17
VII- P.B (5mg/kg)	4.62± 0.34	1.68± 0.88	44.95± 7.23	35.68± 6.24	15.88 ± 2.02	304.71 ± 116.13	6.50± 0.29	9.85± 0.65	47.03± 6.87	12.98± 2.52	26.03± 2.18
VIII- P.B (10mg/ kg)	4.54±0. 35	1.69± 0.72	44.85± 7.15	32.68± 7.26	16.78 ± 2.05	308.73 ± 146.23	6.40± 0.39	9.45± 0.44	47.63± 7.62	12.96± 2.62	26.13± 2.07
IX- P.B (15mg/ kg)	4.21±0. 42	1.46± 0.81	45.75± 7.13	34.48± 7.44	15.99 ± 2.25	308.57 ± 126.13	7.10± 0.43	8.25± 0.74	47.03± 6.83	11.76± 2.56	26.33± 1.27
X- P.B (20mg/ kg)	4.11±0. 34	1.76± 0.58	44.85± 7.33	34.58± 7.22	16.98 ± 1.05	320.74 ± 116.33	7.50± 0.43	7.45± 0.91	46.43± 7.63	11.68± 2.42	25.23± 1.17

Valuesare expressed as mean ± SEM. P.B= *Plasmodium berghei* infected
*p< 0.05
n = 10

4.2 NAPHTHOQUINONE STUDIES

4.2.1 Effects of naphthoquinone on sperm indices in mice

There was a significant reduction in sperm viability, motility and counts (million/ml) with all the concentrations tested when compared with their control counterparts (table 4). Similarly, there were higher decreases in sperm indices in *Plasmodium berghei* treated mice which appear to be augmented by naphthoquinone treatment (table 4).

Table 4: Effects of naphthoquine alone on sperm indices in *Plasmodium berghei* infected and uninfected mice on sperm indices

Treatment groups	Sperm viability (%)	Sperm motility (%)	Sperm count (million/ml)
Grp I-Control (0 mg/kg)	88.33± 4.73	70.33± 1.53	20.75± 2.46
Grp II- 0.1mg/kg naphthoquinone	83.33± 2.52	61.67± 6.43*	13.50± 2.38*
Grp III- 0.5mg/kg naphthoquinone	83.33± 4.62	58.67± 2.89*	13.58± 2.16*
Grp IV-1mg/kg naphthoquinone	79.00± 3.00*	57.67± 6.81*	13.17± 1.01*
Grp V -2mg/kg naphthoquinone	63.00± 2.65*	41.33± 8.33*	9.42± 3.01*
Grp VI-*Plasmodium berghei* infected mice	84.23± 3.02	53.06± 2.05*	20.07 ± 2.32
Grp VII-*Plasmodium berghei* infected + naphto (0.1mg/kg) mice	63.10± 4.65*	40.34± 7.30*	15.16± 1.01*
Grp VIII-*Plasmodium berghei* infected naphto (0.5mg/kg)mice	62.50± 2.14*	39.85± 8.24*	14.10± 1.04*
Grp IX-*Plasmodium berghei* infected naphto (1mg/kg) mice	62.30± 2.75*	39.53± 8.43*	13.13± 1.02*
Grp X-*Plasmodium berghei* infected naphto (2mg/kg) mice	61.02± 2.60*	21.33± 6.33*	12.17± 1.03*

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

4.2.2. Effects of naphthoquinone on reproductive hormones in *Plasmodium berghei* infected and uninfected mice

Naphthoquinone administration to normal male mice and *Plasmodium* infected mice caused significant decrease ($p<0.05$) in serum levels of LH, FSH and testosterone. However this, drug significantly ($p<0.05$) increased serum prolactin level in these mice (Table 5-11).

Table 5:Serum Luteinizing Hormone, Prolactin, Follicle stimulating Hormone and Testosterone levelsin uninfected (Negative control) male mice

Hormones	Values (ng/ml)
Luteinizing Hormone	4.26 ± 0.18
Prolactin	1.55 ± 0.40
Follicle stimulating Hormone	2.84 ± 0.90
Testosterone	4.80 ± 0.74
Cortisol	1.02 ± 0.01

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

Table 6: Serum Luteinizing Hormone, Prolactin, Follicle stimulating Hormone and Testosterone levels in*Plasmodium berghei* infected (Positive control) male mice

Hormones	Values (ng/ml)
Luteinizing Hormone	1.22 ± 0.39
Prolactin	0.40 ± 0.32
Follicle stimulating Hormone	2.26 ± 0.42
Testosterone	2.15 ± 0.19
Cortisol	3.02 ± 0.12

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

Table 7: Serum Luteinizing Hormone, Prolactin, Follicle stimulating Hormone and Testosterone levels in *Plasmodium berghei* infected male mice treated with 0.1mg/kg BW naphthoquinone

Hormones	Values (ng/ml) 0.1 mg/ml naphthoquinone	Values (ng/ml) 1 mg/ml naphthoquinone	Values (ng/ml) 2 mg/ml naphthoquinone
Luteinizing Hormone (LH)	1.40 ± 0.32	0.7 ± 0.32	2.60 ± 0.58
Prolactin (PR)	0.42 ± 0.39	0.36 ± 0.36	1.66 ± 0.54
Follicle stimulating Hormone (FSH)	2.42 ± 0.66	2.02 ± 0.26	3.16 ± 0.36
Testosterone	3.24 ± 0.42	3.52 ± 0.52	2.36 ± 0.42
Cortisol	3.02 ± 0.21	3.04 ± 0.19	3.11 ± 0.23

Valuesare expressed as mean ± SEM
*p< 0.05 n = 10

Table 8: Comparison of mean serum Luteinizing Hormone, Prolactin, Follicle stimulating Hormone and Testosterone levels in*Plasmodium berghei* uninfected (negative control) and *Plasmodium berghei* infected (positive control) male mice

Hormones (ng/ml)	Mean of *P. berghei* uninfected	Mean of *P. berghei* infected
Luteinizing hormone	4.26 ± 0.18	1.22 ± 0.39
Prolactin	1.55 ± 0.40	0.40 ± 0.32
Follicle stimulating hormone	2.84 ± 0.90	2.26 ± 0.42
Testosterone	4.80 ± 0.74	2.15 ± 0.19
Cortisol	1.02 ± 0.01	3.02 ± 0.12

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

Table 9: Comparison of mean serum Luteinizing Hormone, Prolactin, Follicle stimulating Hormone and Testosterone levels in*Plasmodium berghei* infected mice (positive control) and *Plasmodium berghei* unfected mice treated with 0.1mg/kg BW Naphthoquinone.

Hormones (ng/ml)	Mean of *P. berghei* uninfected	Mean of *P. berghei* infected
Luteinizing Hormone	1.22± 0.39	1.40± 0.32
Prolactin	0.40± 0.32	0.42± 0.39
Follicle stimulating Hormone	2.26± 0.42	2.42± 0.66
Testosterone	2.15± 0.19	3.24± 0.42
Cortisol	1.02 ± 0.01	3.02 ± 0.21

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

Table 10:Comparison of mean serum Luteinizing Hormone, Prolactin, Follicle stimulating Hormone and Testosterone levels in *Plasmodium berghei* infected mice (positive control) and *P. berghei* infected mice treated with 1mg/kg BW naphthoquinone.

Hormones (ng/ml)	Mean of *P. berghei* infected	Mean of *P. berghei* infected + naphthoquinone treatment
Luteinizing Hormone	1.22± 0.39	0.70 ± 0.32
Prolactin	0.40± 0.32	0.36± 0.36
Follicle Stimulating Hormone	2.26± 0.42	2.02± 0.26
Testosterone	2.15± 0.19	3.52± 0.52
Cortisol	3.02 ± 0.12	3.04 ± 0.19

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

Table 11**:** Comparison of serum Luteinizing Hormone Prolactin Follicle stimulating Hormone and Testosterone levels in *Plasmodium berghei* infected mice (positive control) and *Plasmodium berghei* infected mice treated with 2.0mg/kg BW naphthoquinone

Hormones (ng/ml)	Mean of *P. berghei* infected	Mean of *P. berghei* infected+ naphthoquinone treatment
Luteinizing Hormone	1.22± 0.39	2.60± 0.58
Prolactin	0.40± 0.32	1.66± 0.54
Follicle Stimulating Hormone	2.26± 0.42	3.16± 0.36
Testosterone	2.15± 0.19	2.36± 0.42
Cortisol	3.02 ± 0.12	3.11 ± 0.23

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

4.2.3 Effect of naphthoquinone on some haematological indices in mice

As shown in table 12, there was a slight increase in the mean Red Blood Cell (RBC) count (5.54 ±0.84) of the group administered with 1.0mg/kg of naphthoquinone than the control group (5.38 ± 1.7) and other groups administered with 0.1mg/kg, 0.5mg/kg and 2.0mg/kg. Decreased White Blood Cell (WBC) counts in all the groups were noted as compared to the control group (3.26±2.1). The group that received 0.5mg/kg dose had a marked decrease in WBC (1.99 ±42) comparing it with the control and other groups. Increased granulocytes were observed in this study as compared with the control group. Packed, Cell Volume (PCV) and Haemoglobin (Hb) were decreased in this study.

The Mean Corpuscular Volume (MCV) was decreased in all the groups compared with the control group. The MCV has a relationship with the RBC and the PCV, since RBC and PCV were decreased, there is every possibility of having a decreased MCV. The granulocytes (GR) were statistically increased at ($P = 0.002$). Lymphocyte and MCH were statistically decreased at ($P = 0.001$). Platelets (Plat) were decreased in all groups except of group II (0.1mg/kg) as against the control group.

In table 12, GR, Lymp, plat, MCV and MCH when analysed using ANOVA, gave the following p-values 0.002, 0.001, 0.019, 0.010 and 0.001 respectively which were statistically significant ($p<0.05$). Others were statistically not significant.

Table 12: Effects of naphthoquinone on haematological indices in mice

Groups	Control	0.1mg/kg	0.5mg/kg	1.0mg/kg	2.0mg/kg
RBC	5.38±1.73	5.06± 0.28	3.98± 0.66	5.54± 0.84	5.12± 0.62
WBC	3.26±2.07	3.06±1.05	1.99± 0.42	2.22± 0.41	2.23± 0.49
GR (%)	28.65±3.57	63.33±5.16	42.10±13.5	29.95±5.20	45.80±17.3
Lymp(%)	62.18±5.34	25.53±1.86	46.50±14.0	54.38±3.02	40.70±14.6
Mono(%)	9.25±3.21	8.70±3.15	9.93±4.70	12.20±5.82	9.85±4.19
Plat	264.50±88.2	282.75±120	81.75±24.5	154.00±22.6	176.00±99.5
Hb	7.20±2.63	6.28± 0.38	6.60±1.91	6.40±2.19	5.80± 0.85
PCV	26.15±8.77	23.08±2.00	20.50±2.88	21.48±2.54	22.78±3.36
MCV	49.00±5.23	45.50±1.91	47.50±5.26	37.75±2.22	44.50±3.70
MCH	13.33±1.50	12.25± 0.79	14.18±1.82	9.73± 0.65	11.48± 0.93
MCHC	27.33±1.38	25.38±2.33	29.03±3.69	25.13± 0.49	25.15± 0.69

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

4.3 EFFECTS OF *PLASMODIUM BERGHEI* ON LIVER ENZYME IN MALE MICE

The resultsshown in table 13 represent mice which were not parasitized, serving as the control group. These resultsare presented as mean ± SEM for the two transaminases. The figure for aspartate transaminase is 632.5 ± 70.6µ/l while for alanine transaminase is 115.7 ± 20 µ/l.

Table 14, shows the result in mean ± SEM of male mice that were parasitized with 0.1ml *Plasmodium berghei* representing 15%. The observed value for aspartate transaminase is 655.2 ± 34.8 µ/l while that of alanine transaminase is 130.6 ± 8.5µ/l. A look at the observed result reveals a slight increase in the values of Aspartate and Alanine transaminases.

Table 15, shows the result in mean ± SEM of male mice that were parasitized with 0.2ml *Plasmodium berghei* representing 30%.

The observed result for aspartate transaminases is 667.3 ± 62.6µ/l while the alanine transaminase is 134.9 ± 9.4µ/l. Table 17 shows the mean values for aspartate and alanine transaminases. This showed that a steady rise in values of the two transaminases was observed from the control, 0.1ml parasitized mice and 0.2ml parasitized mice.

Finally, table 18 shows the comparison between 0.1ml and 0.2ml parasitized mice for both aspartate and alanine transaminases.

Table 13:Levels of AST and ALT in Negative control male mice

Enzyme	Concentration
AST	632.5 ± 70.6µ/l
ALT	115.7 ± 20.0µ/l

Table 14:Levels of AST and ALT in male mice that were infected with0.1 ml *Plasmodium berghei*

Enzyme	Concentration ¯
AST	655.2 ± 34.8µ/l
ALT	130.6 ± 8.5µ/l

Table15: Levels of AST and ALT in male mice that were parasitized with 0.2ml *Plasmodium berghei*

Enzyme	Concentration
AST	667.3 ± 62.6µ/l
ALT	134.9 ± 9.4µ/l

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

Table 16: Comparison of serum levels of ALT and AST in 0.1ml parasitized and non-parasitized male mice

Enzyme	*P. berghei* infected	Uninfected
AST	632.5± 70.6	655.2±34.8
ALT	115.7± 20.0	130.6± 8.5

Table 17: Comparison of serum levels of ALT and AST in 0.2ml parasitized and non-parasitized male mice

Enzyme	*P. berghei* infected	Uninfected
AST	632.5± 70.6	667.3± 62.6
ALT	115.7± 20.0	134.9± 9.4

Table 18:Comparison of serum levels of ALT and AST in 0.1ml and 0.2ml parasitized male mice

Enzyme	0.1ml Parasitized	0.2ml Parasitized
AST	655.2±34.8	667.3± 62.6
ALT	130.6± 8.5	134.9± 9.4

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

•

KEYS

Control

0.1ml parasitized

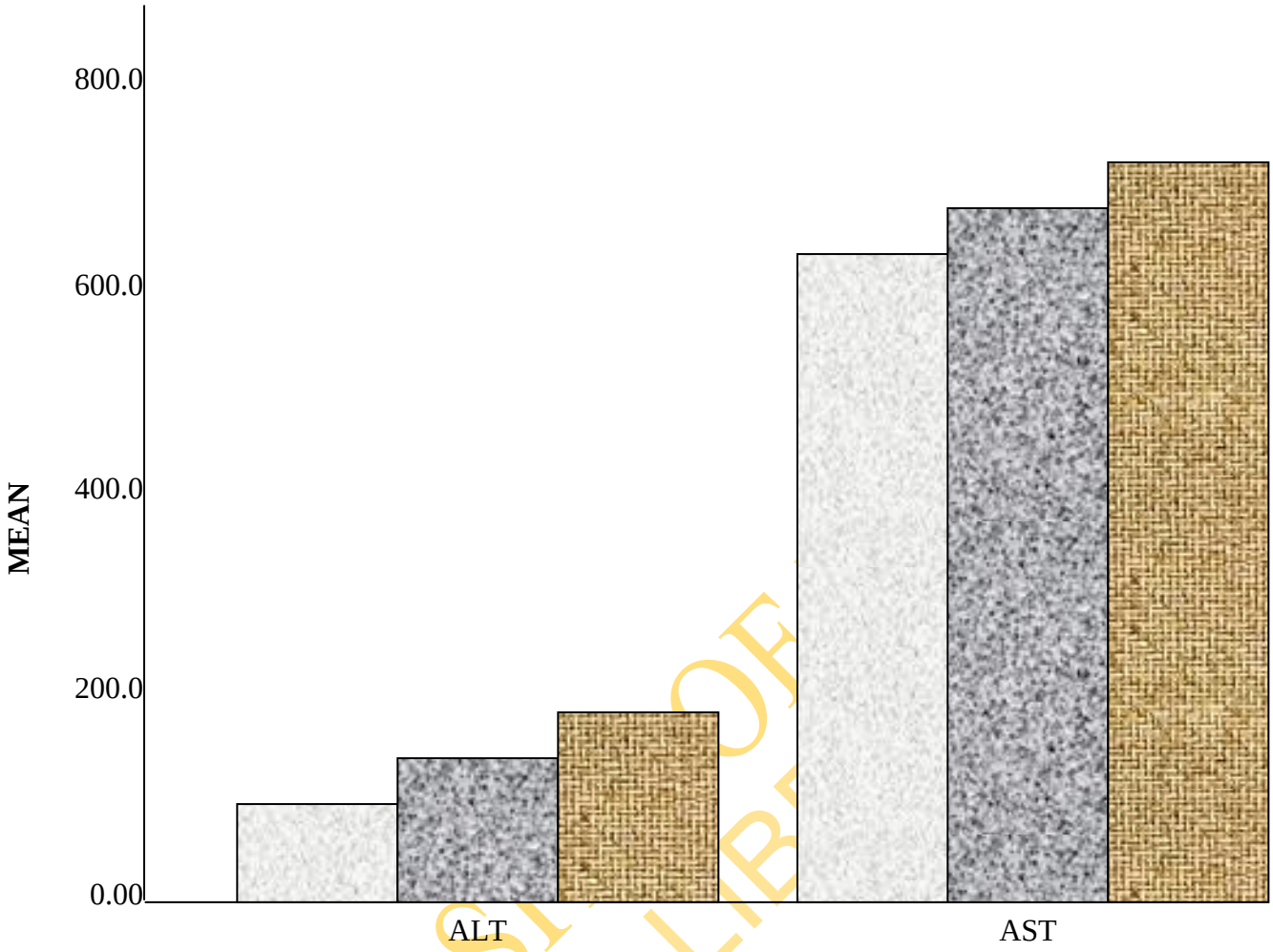

Figure 3:Comparison of serum levels of ALT and AST in control, 0.1ml and 0.2ml paratized male mice.

4.4. ANTIOXIDANT ENZYMES IN *PLASMODIUM BERGHEI* INFECTED MICE TREATED WITH NAPHTHOQUINONE

Tables 19 and 20 showed that naphthoquinone and chloroquine produced a dose-dependent reduction in the SOD content of the serum that was not significant when compared with the

control. There was also no significant difference in the catalase and MDA activities when compared with the control. Furthermore neither *Plasmodium berghei* alone or under treatment with naphthoquinone and chloroquine produced any significant effect in the antioxidant enzymes.

Table 19: Anti-oxidant enzymes in *Plasmodium berghei* infected mice treated with Naphthoquinone

Treatment groups	SOD	CATALASE	LPO MDA units/mg Protein
Grp I-Control (0 mg/kg)	2.6 ± 0.80	78.31± 4.81	0.42±0.02
Grp II- 0.1mg/kg	2.8 ± 0.40	70.20 ± 3.81	0.37±0.03

naphthoquinone			
Grp III- 0.5mg/kg naphthoquinone	3.6 ± 0.50	93.20 ± 3.42	0.36±0.03
Grp IV-1mg/kg naphthoquinone	3.9 ± 0.40	90.34 ± 3.44	0.33±0.02
Grp V -2mg/kg naphthoquinone	4.5 ± 0.36	96.24 ± 4.00	0.21± 0.02
Grp VI-*Plasmodium berghei* infected mice	1.9 ± 0.23	79.21± 3.82	0.38 ± 0.04
Grp VII-*Plasmodium berghei* infected + naphthoquinone (0.1mg/kg) mice	1.8 ± 0.33	80.13 ± 3.49	0.35 ± 0.02
Grp VIII-*Plasmodium berghei* infected naphthoquinone (0.5mg/kg)mice	1.9 ± 0.21	82.13± 3.28	0.33 ± 0.03
Grp IX-*Plasmodium berghei* infected naphthoquinone (1mg/kg) mice	1.9 ± 0.22	84.15± 3.17	0.30 ± 0.03
Grp X-*Plasmodium berghei* infected naphthoquinone (2mg/kg) mice	2.1 ± 0.25	86.23 ± 3.25	0.29 ± 0.02

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

Table 20: Effects of *Plasmodium berghei* treated and untreated with chloroquine on serum anti-oxidants enzymes in male mice

Treatment groups	SOD	CATALASE	LPO MDA units/mg Protein
Grp I-Control (0 mg/kg)	2.6 ± 0.80	78.31± 4.81	0.42±0.02

Grp II- 5mg/kg chloroquine	1.9 ± 0.43	79.20 ± 2.81	0.38±0.03
Grp III- 10mg/kg chloroquine	1.7 ± 0.40	84.20 ± 3.52	0.37±0.04
Grp IV-15mg/kg chloroquine	2.7 ± 0.30	88.34 ± 3.54	0.35±0.03
Grp V -20mg/kg chloroquine	2.3 ± 0.26	94.84 ± 5.10	0.36± 0.02
Grp VI-*Plasmodium berghei* infected mice	1.9 ± 0.23	79.24± 3.72	0.38 ± 0.05
Grp VII-*Plasmodium berghei* infected + chloroquine (5mg/kg) mice	1.8 ± 0.43	81.23 ± 3.51	0.36 ± 0.02
Grp VIII-*Plasmodium berghei* infected chloroquine (10mg/kg)mice	2.0 ± 0.31	83.23± 3.17	0.34 ± 0.03
Grp IX-*Plasmodium berghei* infected chloroquine (15mg/kg) mice	2.0 ± 0.22	84.47± 3.18	0.30 ± 0.03
Grp X-*Plasmodium berghei* infected chloroquine (20mg/kg) mice	2.1 ± 0.26	86.43 ± 3.45	0.30 ± 0.02

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

TESTICULAR HISTOLOGY

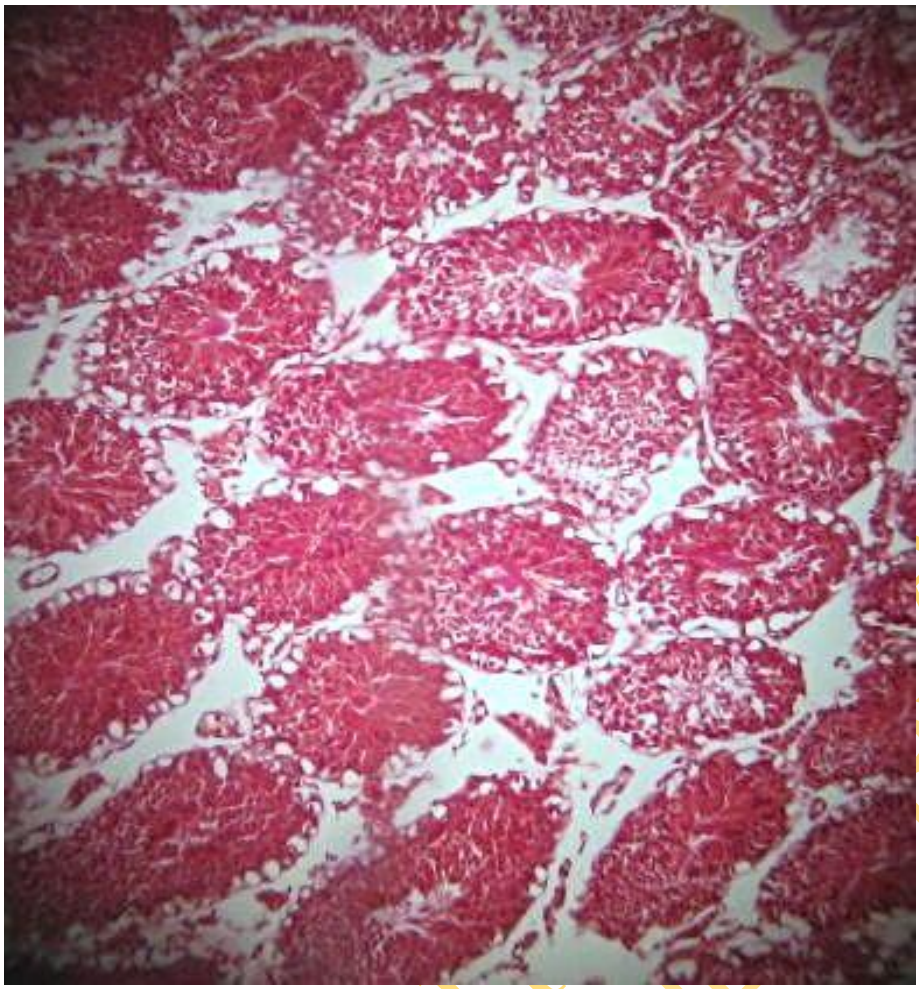

Plate 1: Photomicrograph of testis of control male mouse (H&E, X40)

The size of the seminiferous tubule were predominantly small with few large elongated seminiferous tubule seen to contain more mature spermatids evidence by the number of flagella found in the lumen. The amount of spermatogonia also seen to vary with the size of the tubule. The Sertoli cells are very numerous. The interstitial cells are generally unremarkable.

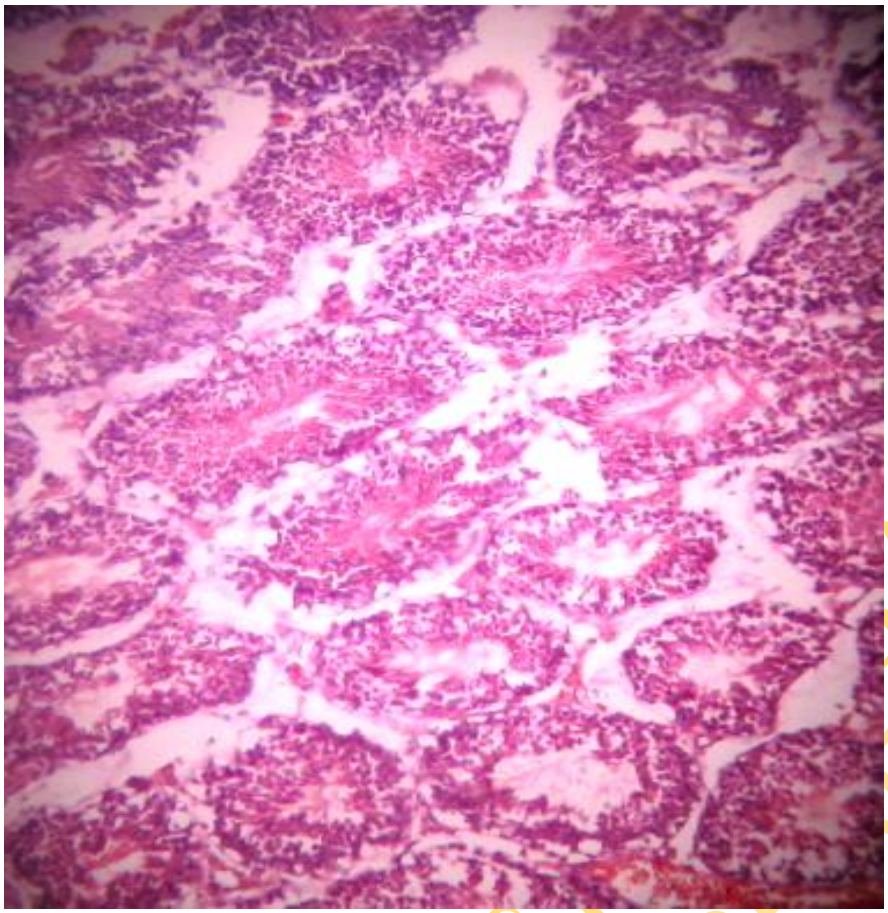

Plate 2: Photomicrograph of testis of uninfected mouse treatedwith 2.0mg/kg/bodyweight of Naphthoquinone (H&E, X40).

Section of testes treated with naphthoquinone show a general distortion of the tubular shape. There are also very few large seminiferous tubule. The germinal epithelia are thick with very small lumen. Connective tissue between the tubule is moderate. The amount of the spermatids in the lumen of the tubule appear to be much but can't not be properly accessed because of the reduce space.

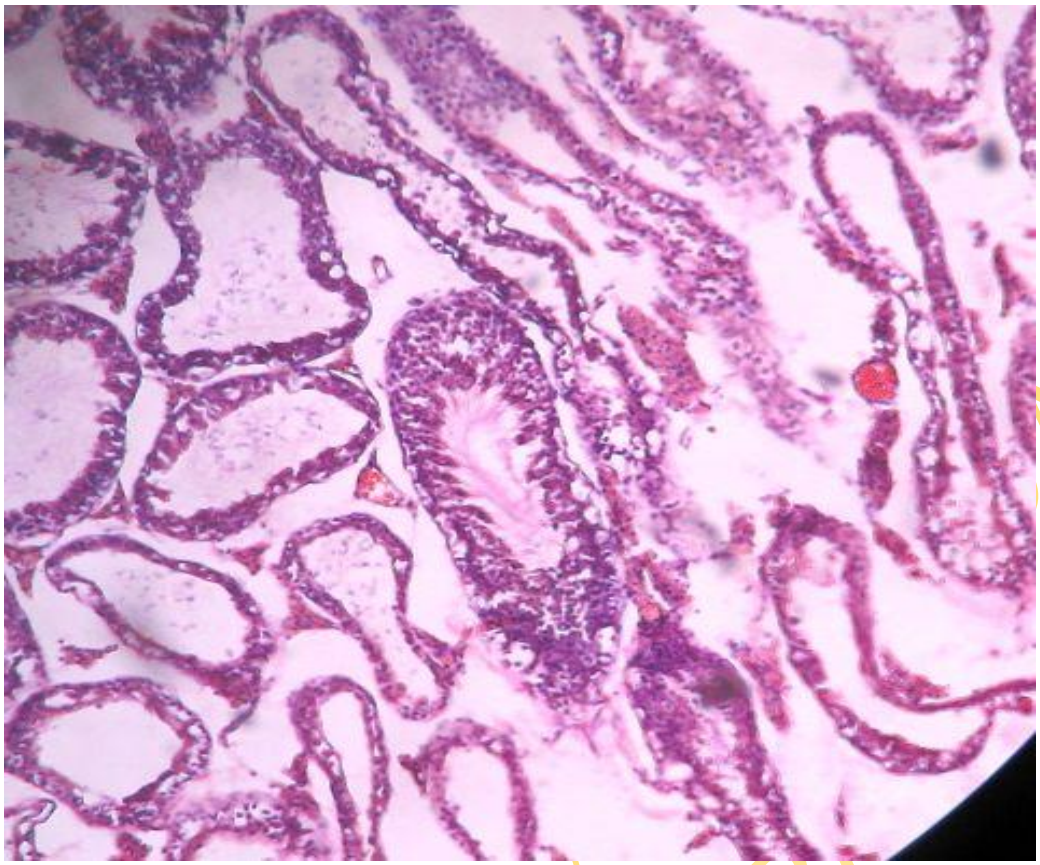

Plate 3: Photomicrograph of testis of infected mouse treated with 2.0mg/kg/ body weight of Naphthoquinone (H&E, X40)

Section of testes treated with naphthoquinone highest dose (2 .0 mg/kg body weight/day) show seminiferous tubule with thin germinal epithelium and very large lumen containing few but vary mature sperm cell. The tubules are generally large. The section gives an impression of tubule whose epithelium is eroded thereby most likely diminishing spermatogenesis.

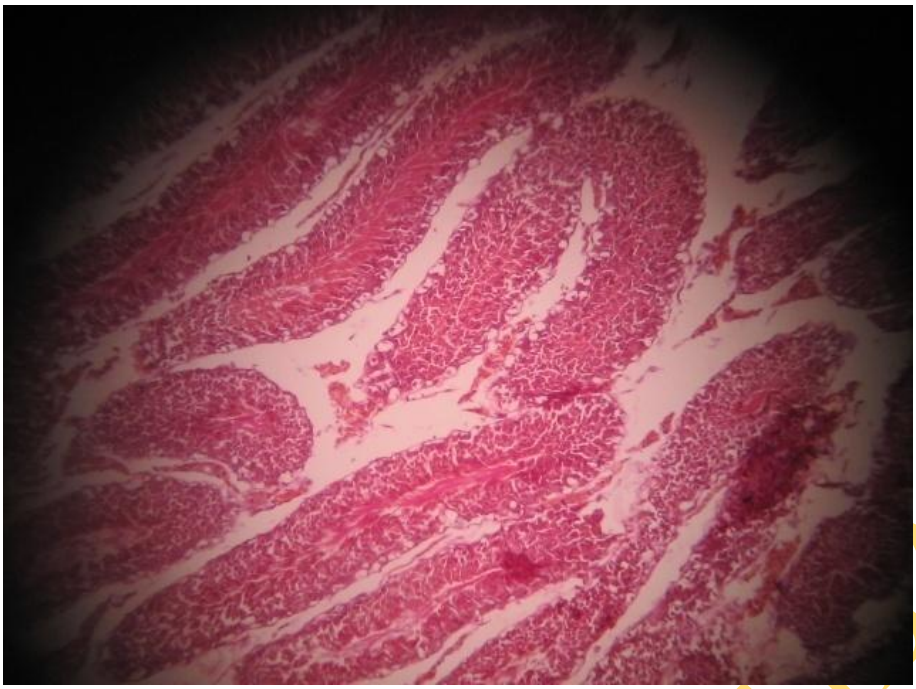

Plate 4: Photomicrograph of testis of mouse infected with *Plasmodium berghei* (positive control) (H&E, X40)

Section shows many larger and elongated seminiferous tubules packed with lumen and spermatid as indicated by flagella. The cells of these tubules tend to take up more stains than the smaller ones suggesting increased cellular activities. Sertoli cells are widely distributed at the edges of the tubules. Difference in spermatogenic activities does not seem to be remarkable from that of the controls

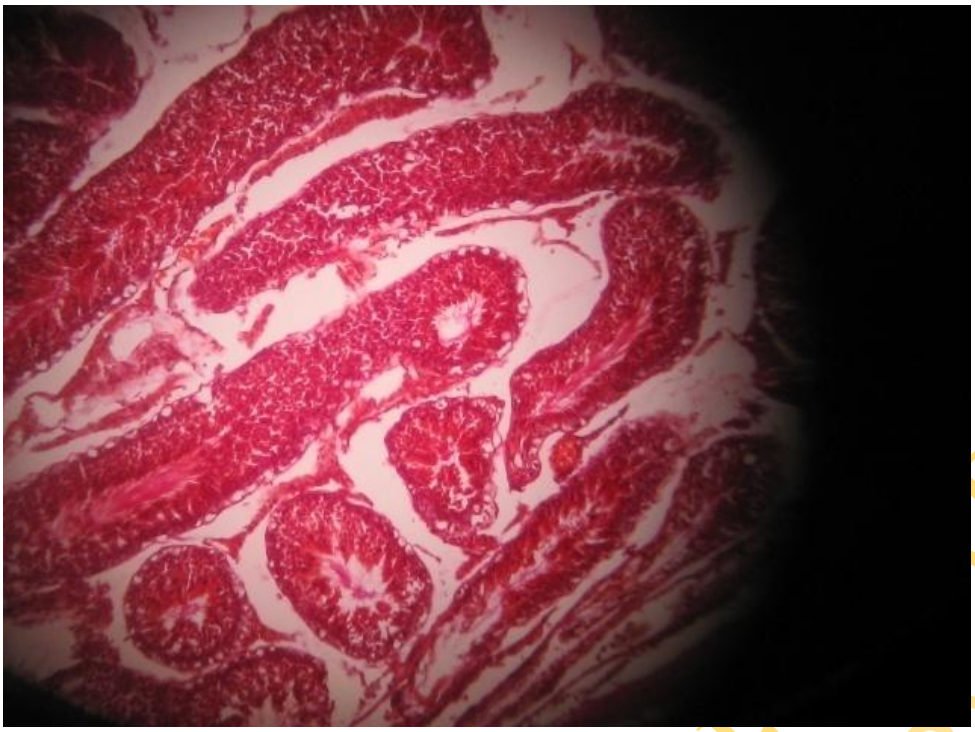

Plate 5: Photomicrograph of testis of mouse treated with chloroquine (H&E, X40)

Section of testes shows large but congested seminiferous tubules most of which contains moderate amount of spermatids. The interstitial spaces are larger which may be as a result of eodema or a processing artifact. The reason for the tightness of the seminiferous tubules is not clear but may have an effect on spermatogenesis if it was *in vivo*.

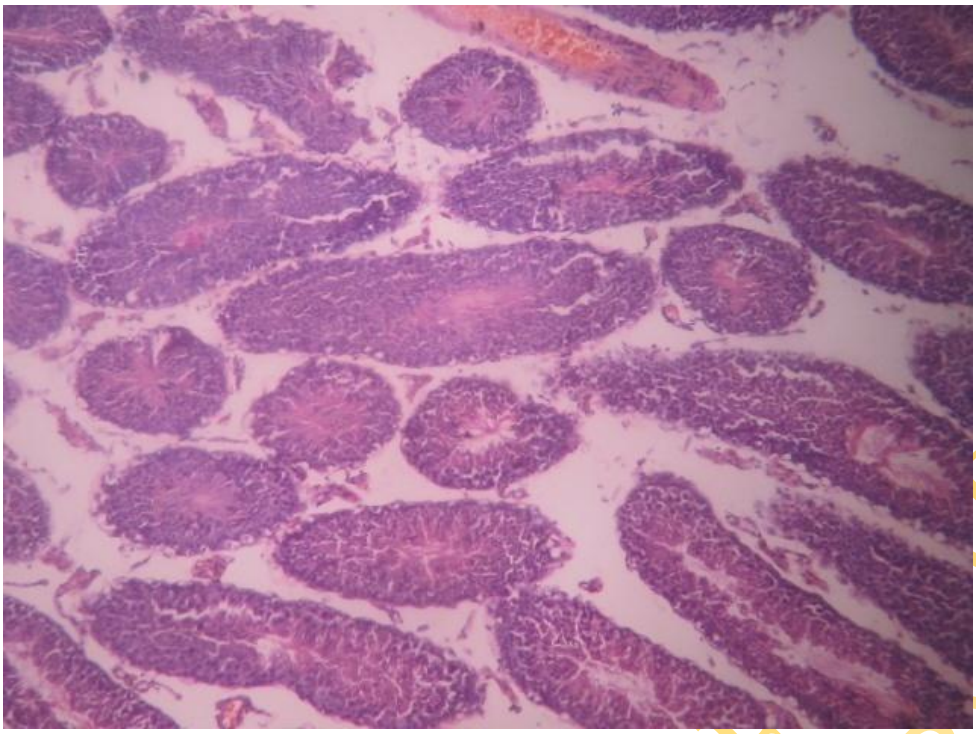

Plate 6: Photomicrograph of testis of mouse infected and treated with 5mg and 10mg/kg of chloroquine (H&E, X40)

Section of the testes generally appears robust with active spermatogenesis and the relatively even distribution of small, medium and large seminiferous tubules all of which contain an appreciable amount of spermatid. The connective tissues between the tubules are moderate and generally unremarkable. The general impression is one of a normal section.

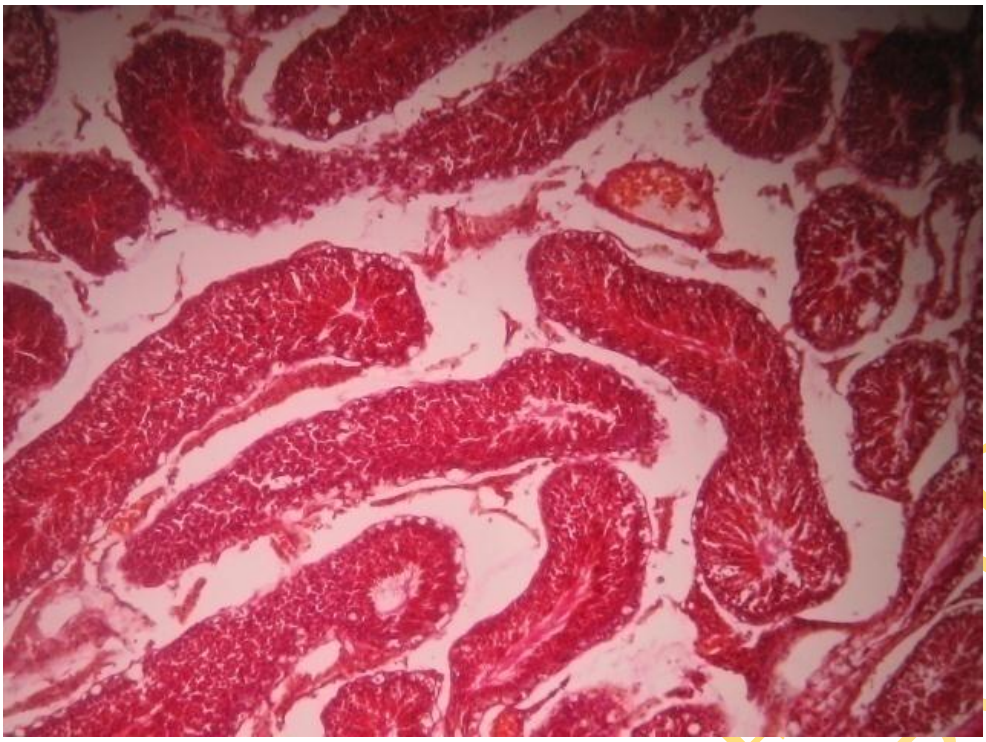

Plate 7: Photomicrograph of testis of mouse infected and treated with 15mg/kg of chloroquine (H&E, X40)

Section of testis shows large seminiferous tubules each with a fairly large lumen and relatively fewer spermatids. Interstitial spaces are large. Sertoli cell are not so obvious especially in the large seminiferous tubules. Connective tissue between the tubules is scanty so there is a general impression of lowered testicular activities.

4.6 OTHER VITAL MEASUREMENTS IN THIS STUDY

Other vital measurements that were carried out in this study include body temperature, body weight and organ weight.

Naphthoquinone and body temperature in male mice

As shown in tables 21 and 22 neither naphthoquinone nor chloroquine produced any effect on body temperature of the uninfected mice. However each of the two drugs produced significant reduction in body temperature of the *Plasmodium berghei* infected mice (Tables 21 and 22).

Table 21: Body temperature (^{0}C) of *Plasmodium berghei* infected and Naphthoquinone treated Male Mice

Treatment groups	Day 1	Day 7	Day 14
Grp I-Control (0 mg/kg)	34.7	34.6	34.7
Grp II- 0.1mg/kg naphthoquinone	34.8	34.7	34.7
Grp III- 0.5mg/kg naphthoquinone	34.8	34.7	34.7
Grp IV-1mg/kg naphthoquinone	34.7	34.6	34.8
Grp V -2mg/kg naphthoquinone	34.8	34.7	34.7
Grp VI-*Plasmodium berghei* infected mice	36.1	34.8	34.7
Grp VII-*Plasmodium berghei* infected + naphthoquinone (0.1mg/kg) mice	36.0	34.7	34.7
Grp VIII-*Plasmodium berghei* infected naphthoquinone (0.5mg/kg)mice	35.8	34.7	34.8
Grp IX-*Plasmodium berghei* infected naphthoquinone (1mg/kg) mice	35.8	34.7	34.7
Grp X-*Plasmodium berghei* infected naphthoquinone (2mg/kg) mice	35.9	34.7	34.7

Valuesare expressed as mean ± SEM
*p< 0.05 n = 10

Table 22: Body temperature (^{0}C) of *Plasmodium berghei* infected and Chloroquine treated Male Mice

Treatment groups	Day 1	Day 7	Day 14
Grp I-Control (0 mg/kg)	34.8	34.7	34.7
Grp II- 5mg/kg chloroquine	34.9	34.9	34.8
Grp III- 10mg/kg chloroquine	35.0	35.0	34.9
Grp IV-15mg/kg chloroquine	35.0	34.9	34.8
Grp V -20mg/kg chloroquine	34.9	35.0	34.9
Grp VI-*Plasmodium berghei* infected mice	36.2	35.0	34.9
Grp VII-*Plasmodium berghei* infected + chloroquine (5mg/kg) mice	36.1	34.9	35.0
Grp VIII-*Plasmodium berghei* infected chloroquine (10mg/kg)mice	36.2	35.0	34.9
Grp IX-*Plasmodium berghei* infected chloroquine (15mg/kg) mice	36.0	34.9	34.8
Grp X-*Plasmodium berghei* infected chloroquine (20mg/kg) mice	36.1	34.9	34.7

Valuesare expressed as mean ± SEM
*p< 0.05
n = 10

CHAPTER FIVE

5.0 DISCUSSION

In this study, a significant reduction in sperm viability, motility and counts in the chloroquine treated mice was observed. The reduction in sperm molity and viability is in accordance with the known antifertility effect of chloroquine and is in accordance with the findings of Adeeko and Dada (1998). Sperm counts were however not in accordance with these findings because there is a decrease in sperm count even in minimum concentration of chloroquine (5mg/kg) in this research.

The reduction in sperm motility and viability observed with chloroquine treatment in this research suggest that chloroquine was able to penetrate into the testes. Earlier studies had shown that chloroquine was concentrated in the testes of guinea pigs, (Grundman and Vrublousky, 1997). Adeeko and Dada (1994) also showed that chloroquine was secreted in human semen. The decrease in sperm motility caused by chemical agents had earlier been attributed to their ability to permeate the blood-testes barrier (Baldessarini, 1980). The marked effect seen might be because of rapid absorption of chloroqine in the body.

Naphthoquinone was also observed to suppress male fertility indices. In this research marked reduction in sperm viability, motility and count was seen. According to Dinnen and Ebisuzaki, (1997), Naphthoquinone is an antiproliferative agent. The mechanism of its antimitiotic effect is due to mitochondrial and phosphorylation inhibition (Santana *et al*, 1968), antagonism of vitamin K reactions (Dinen and Ebisuzaki, 1997). This reduction in fertility indices suggests that naphthoquinone also crosses the blood-testes barrier. Rita and Martha (2007) suggest reproductive toxicity of lapachol- a naphthoquinone extracted from plants of the genus Tabebuia, indicating seminal vesicle as the possible target organ.

Both antimalarial drugs tested have a marked effect on reproductive indices of mice. Moreover, all the concentrations tested showed a marked effect. This suppression of reproductive indices even though reversible at normal therapeutic dose may become a problem with indiscriminate use of these antimalarial drugs such as chloroquine.

According to Lauralee (2001) the average sperm count fell from 113 million/ml of semen in 1940 to 66 million in 1990, and the volume of single ejaculate has declined from 3.40ml to 2.75ml. This means that men on the average are now ejaculating less than half the number of sperms as men did 50 years ago. A drop from more than 380 million sperm to about 180 million sperm per ejaculate was also observed. Furthermore, the number of motile sperm has also dipped. In summary, both chloroquine and naphthoquinone exhibit a marked impairment of sperm motility, viability and counts and both drugs showed a dose dependant effect on these parameters, that is, as the dose increases, the reproductive parameters (viability motility and counts of sperm) decreases.

Naphthoquinone treatment in mice caused regressive histological changes distortion of the seminiferous tubule resulting in the suppression of spermatogenesis. Furthermore, the antispermatogenic effects induced by naphthoquinone in testes of mice were reversible.

The mechanism by which naphthoquinone causes antispermatogenic effect is not well understood. Studies of Uematsu (1966) suggest that naphthoquinone treatment causes suppression of spermatogenesis by acting directly on the seminiferous epithelium, and that this action is not mediated via the hypophysis.

Further, Hagenas *et al (*1978) have shown that naphthoquinone acts directly on germ cells and thereby causes arrest of spermatogenesis. In the present study, section of the testes show seminiferous tubule with thin germinal epithelium and very large lumen containing

few but vary mature sperm cell. The tubules are generally large. The section gives an impression of tubule whose epithelium is eroded thereby most likely diminishing spermatogenesis.

It is pertinent to mention here that Hoffer (1983) has also reported occurrence of intraepithelial vacuoles in affected seminiferous tubules in rat testes after gossypol treatment, and that these vacuoles occurred primarily in the Sertoli cells. Such vacuoles are also reported to occur in the Sertoli cells after several kinds of testicular injuries, and they have, however, often been interpreted as a nonspecific reaction of these cell Fawcett (1975). Thus, it is difficult to say in the mice as to how naphthoquinone treatment induces antispermatogenic effect in the testes, though the possibility of a direct action of the drug on the germ cells can not be ruled out.

The rate of consumption of food by the infected animals is low compared to the control groups; this might be due to the malaria parasite. The variation of temperature of all the animal groups is still the same before and after administration of chloroquine. Interstitial cells, sertoli cells and seminiferous tubules have been observed following treatment of mice with test doses of chloroquine. In infected and treated mice it was observed that the seminiferous tubules were very large containing large lumen and relatively fewer spermatids and also showed that Sertoli cell are not so obvious especially in the large seminiferous tubules.

These results are in agreement with reports from other investigator who observed that chloroquine caused disruption of spermatogenesis due to insufficient production of androgen by leydig cells, (Ebong *et al,* 1999). It was also reported that the regression observed in sertoli and leydig cells after treatment may suggest that chloroquine is toxic to the sertoli cell and leydig cell. The toxicity is probably mediated via effect on the anterior pituitary (Okanlawon and Dyn, 1996). In infected but not treated mice, it also showed many large and enlongated seminiferous

tubules packed with lumen, spermatid and sertoli cells are widely distributed at the edge of the tubules.

This study has also revealed that chloroquine treatment in mice lowered the testicular activities and the difference in spermatogenic activities does not seem to be remarkable. This study also showed that *Plasmodium berghei* infection has effect on the liver enzymes of male mice by causing a rise in both aspartate and alanine transaminases with a more increase in aspartate transaminases, which is due to cytoplasmic membrane damage of the liver cells. The increase in the liver enzymes indicates that there is liver injury, due to the merozoites of the malaria parasite invading the liver cells. The process is likely causing a rupture in the cytoplasmic membranes of the liver, which corresponds with the study of Adachi et al in 2001. It was observed that the *Plasmodium berghei* infection caused a rise in the liver enzymes and the rise was according to the degree or level of parasitaemia in the mice, which is in line with the study of Ute Frevert (2005).

There was a slight increase in the mean Red Blood Cell (RBC) count of the group administered with 1.0mg/kg of naphthoquinone than the control group and other groups administered with 0.1mg/kg, 0.5mg/kg and 2.0mg/kg. Naphthoquinone share the same structure with vitamin K (Dinen and Ebisuzaki, 1997)and the reason for the increase might be because of having the property that enhances erythropoiesis.

A decreased White Blood Cell (WBC) count in all the groups was noted as compared to the control group. The group that received 0.5mg/kg dose had a marked decrease in WBC comparing it with the control and other groups. There is every likelihood that the 1,4-naphthoquinone may have effect on the WBC of these mice.

Increased granulocytes were observed in this study as compared with the control group. The reason may be because, the granulocytes (Neutrophil, Eosinophil and Basophil) were responding to the drug and there was activation of them from the storage pool (tissue).

Packed, Cell Volume (PCV) and Haemoglobin (Hb) were decreased in this study and that 1, 4- naphthoquinone causes hemolytic anaemia in animals and that the hemolysis is in a dose-dependent manner.

The Mean Corposcular Volume (MCV) was decreased in all the groups compared with the control group. The MCV has a relationship with the RBC and the PCV, since RBC and PCV were decreased, there is every possibility of having a decreased MCV.

It was noticed that, there was no significant difference between the different concentrations in RBC, WBC, Monocyte (Mono), Hb, PCV and Mean Corposcular Haemoglobin Concentration (MCHC).

The granulocytes (GR) were statistically increased. Lymphocyte and MCH were statistically decreased, Platelet (Plat) were decreased in all the groups exception of group II (0.1mg/kg) as against the control group. This is because; naphthoquinone has antiplatelet properties (Yuk *et al*, 2001).

Effect of naphthoquinone on haematological parameters (RBC, WBC< Plat, Hb and PCV) was carried out using different concentrations (0.1mg/kg, 05.mg/kg, 1.0mg/kg and 2.0mg/kg). A comparison of their alteration patterns with the control was also observed.

There was a decreased in the number of RBC with increase in concentration of chloroquine. This suggests a depression in haemapoctic process as the dose is increased. This report is similar to that of Mbajiorgu *et al* (2007), where a significant reduction of RBC was observed in unparasitised rats that were administered chloroquine within intervals.

The WBC count also showed the same pattern with that of RBC, where the mean values were decreased with increase in concentration of the drug. This could be due haemapoetic response and a sharp response by the immune system in order to defend the body. The decrease in total leucocyte count at high dose (>10mg) could be due to toxic effect of the drug on leucocytes which was reported by Adelusi *et al* (1982) to concentrate in leucocytes, thus at higher doses could cause their destruction or suppress the haemapoetic system.

In granulocytes, the test groups mean values were shown to be higher than the control. A report by MacCluskey (1991) showed that, high dose of chloroquine causes agranulocytosis which is similar to this finding. Chloroquine is concentrated invivo by lymphocytes where it is known to inhibit antigen processing. Their high participaton to fight any antigen could lead to their possible reduction in number with increase in the concentration while the increase in monocyte could be due to a response by the immune system against the drug.

The mean platelets values were shown to decrease with increase in the concentration of the drug this might be due to the ability of Chloroquine to cause relaxation of smooth muscle and inhibition of platelet activation. This finding contradicts the work of Osim *et al* (1999), who reported that chloroquine prolongs the life span of platelet aggregation thereby increasing the number of circulating platelets without any effect on bleeding time. A decreased platelet number was observed in the test groups and a very sharp decrease in group V. This could probably be due to a strong effect of the high concentration of the drug.

Chloroquine did not affect the morphology of the cells in all concentrations, but polychromatic cells and reactive lymphocytes were seen. This indicated a response by the erythropoetic system to produce more cells due to destruction of the cells and the toxic effect of the drug lead to presence of reactive lymphocytes.

PT of naphthoquinone at concentration of 0.1mg/kg was reduced in time when compared with that of control which agrees with the studies of James *et al*, (2005). As the concentrations increases along 0.5mg/kg, 1.0mg/kg the PT also increased. There was tremendous increase in PT with 2.0mg/kg concentration of the drug. This result can be attributed to the effect of naphthoquinone in blood coagulation. Similarity with vitamin K in structure and to an extent in function contributed to the lowering of PT lower dose and effect of the drug at higher dosage resulting to the increased.

When chloroquine was used, the PT increased steadily as the concentration of the drug was increased as compared with the control. At concentrations of 5mg/kg, 10mg/kg, 15mg/kg and 20mg/kg the mean PT was increased in a dose dependent manner.

The APTT results obtained for the control and different concentrations were all within the normal range of with the exception of 5mg/kg concentration which showed apparent but in significant difference with mean as compared to control (35.33±0.56). AT higher concentrations of 10mg/kg, 15mg/kg and 20mg/kg, the results were within the normal range. The pattern of increase at lower dose is unclear owing to the fact that chloroquine binds to phospholipids.

In this study, it has been discovered that naphthoquinone has effect on both PT and APTT with significant increase statistically in the results. On the contrary, chloroquine has no effect on the coagulation system because there was no statistical significant difference in the results of both PT and APTT. From the post hoc (Duncan) naphthoquinone in the concentration of 2mg/kg was responsible for the increase in both PT and APTT. Generally, the PTR of chloroquine showed apparent but insignificantly increase at lowest dose (5mg/kg) when compared to PTR of naphthoquinone at its lowest dose (0.1mg/kg). At higher doses, PTR of naphthoquinone were higher than that of chloroquine. At toxic doses, the APTT, PT and PTR of chloroquine was the

same as those of 10mg/kg and 15mg/kg concentrations , while PT, APTT and PTR of mice that received the toxic dose of naphthoquinone showed about 60% increase compared to those at lower doses.

Therefore, it can be concluded from this findings that chloroquine has no effect on the coagulation system. This result disagrees with the findings from work done by Ramanathan *et al*, (2002)which observed that chloroquine obrogated clotting of plasma by inhibiting calcium chloride and thrombin.

Also, the fact that high dose of naphthoquinone has effect on coagulation system also disagrees with the findings of Yuk *et al*, (2001) that a newly synthesized naphthoquinone NQ304 had no effect on PT and APTT but prevented thrombosis due to its ability to prevent platelet aggregation. Prolonged PT and APTT by toxic dose of naphthoquinone may be due to hyper activation of vitamin K depedent coagulation factors which may have lead to their consumption *in vivo*.

Conclusion and Contributions to Knowledge

a. 1, 4 –naphthoquinone was shown to have antifertility effects in male mice

b. 1, 4 –naphthoquinone's male antifertility effects could be due to reduction of androgen secretion as the drug caused significant decrease in testosterone.

c. Oxidative stress mechanism, which is usually implicated in several pathological processes is not involved in the antifertility effects of 1, 4 –naphthoquinone.

d. 1, 4 –naphthoquinone has similar biochemical and haematological properties to chloroquine.

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