Effects of novel chloroquine formulation on blood glucose concentration, renal and cardiovascular function in experimental animal paradigms

by

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DECLARATION

I, **Pretty Murambiwa**, hereby declare that the dissertation entitled

"Effects of novel chloroquine formulation on blood glucose concentration, renal and cardiovascular function in experimental animal paradigms" is a result of my own investigation and research and that it has not been submitted in part or in full for any other degree or to any other university. Where use was made of the work of others, it is duly acknowledged in the text.

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Dedication

I dedicate this work to my Lord, Savior, Redeemer and Sanctifier, Jesus Christ,

to my beloved best friend, soulmate and husband Munyaradzi Murambiwa, to

my amazing and much cherished daughter Tinevimbo Murambiwa and to my

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Zenzo Ncube. Lalani ngokuthula, ngiyanithanda nonke.

ABSTRACT

Malaria disease poses a serious global health burden as recent reports have indicated that nearly half of the world's population is at risk (WHO 2008). The World Health Organization (WHO) Expert Consultative Team has reported that 90% of all malaria deaths occur in Sub Saharan Africa. (WHO, 2008). Despite the numerous global efforts to control and manage the disease, through various ways, use of chemotherapeutic agents continues to be the major intervention strategy in the control of malaria. The WHO recommended use of Artermisinin combination therapy (ACT) has been hampered by an imbalance between demand and supply in the poor socioeconomically challenged rural populations of Sub Saharan Africa, the epicenter of malaria infection. Chloroquine (CHQ), therefore, continues to be used in most malaria endemic areas in developing countries despite development of *P. falciparum* resistance to the drug (WHO, 2006). Oral administration is the major delivery route for CHQ. However, CHQ is a bitter drug, with an inconvenient dosing schedule leading to incomplete courses of therapy by most malaria patients. Oral CHQ administration is also associated with adverse effects in various organ systems resulting from deposition of CHQ in these organs to elicit impairment of glucose homeostasis, renal and cardiovascular function. Alternative methods of CHQ administration such as transdermal delivery have, therefore, been suggested, in an effort not only to avoid the bitter taste, but also to modify the dosing schedule, which may improve patient comfort and compliance. Transdermal delivery of CHQ via an amidated matrix patch, which is envisaged to ensure a slow, controlled and sustained release of therapeutic concentrations of CHQ, may circumvent the previously reported adverse effects of oral CHQ. It is against this background that the current study compared the effects of transdermal CHQ patch and oral chloroquine in the management of malaria as assessed by the ability to clear parasites of *P. berghei* infected rats. The other aims were to investigate and distinguish between the patho physiological effects of malaria and CHQ treatments on blood glucose and plasma insulin concentration, renal and cardiovascular function in male Sprague-Dawley rats.

To investigate and distinguish between the pathophysiological effects malaria infection and CHQ treatments on blood glucose homeostasis, renal and cardiovascular function markers, separate groups of non infected and *P. berghei* infected male Sprague Dawley rats (90g-150g) were used. The animals were treated twice daily with oral CHQ (60mg/kg) and a once off transdermal delivery of CHQ via topical application of pectin CHQ matrix patch (53mg/kg) in a 21 day study divided into pre treatment, (days 0-7) treatment (days 8-12) and post treatment (days 13-21) periods. The animals were housed individually in metabolic cages for the duration of the study. Treatment was for 5 consecutive days. Measurements of body weight, food and water intake, mean arterial pressure (MAP), blood glucose concentration, % parasitaemia, haematocrit, and 24 hour urine volume, Na^+, K^+, urea and creatinine outputs were done every day during the treatment period, and every third day during the pre and post treatment periods. Separate groups of non fasted conscious animals (n=6) were sacrificed on days 0, 7, 8, 9, 10, 12, 14 and 21, at 24 hours after the last treatment for oral CHQ administration and after a once off patch application on the first day of treatment. The plasma obtained was assayed for plasma insulin, lipid profile parameters and plasma Na^+, K^+, urea and creatinine. The harvested liver and gastrocnemius muscle were used for determination of glycogen concentration.

The current study has demonstrated the sustained controlled release of CHQ from the pectin matrix patch, demonstrating the therapeutic ability to clear *P. berghei* malaria parasites from systemic circulation. Malaria infection and oral CHQ treatment exhibited blood glucose lowering effects which were circumvented by topical application of the pectin CHQ matrix patch.
Oral CHQ elevated hepatic glycogen concentration through mechanisms that are still to be elucidated. Topical application of CHQ via pectin matrix patch did not alter hepatic and gastrocnemius muscle glycogen concentrations. Malaria infection and oral CHQ delivery reduced food intake, water intake and % body weight changes of the animals as well as inducing natriuresis, reduced urine output and increased urinary creatinine outputs. Malaria infection was also shown to elicit hyperkalaemia and kaliuresis in experimental animals. Hypotensive effects of malaria infection and oral CHQ delivery were also demonstrated in the current study. Malaria infection and oral CHQ delivery elevated plasma total cholesterol and LDL-c as well as reduction in the cardio protective particle, plasma HDL-c, concentrations. Topically delivered

CHQ via pectin CHQ matrix patch did not evoke any such alterations, suggestive of its ability to circumvent the observed adverse effects of oral CHQ delivery due to sustained, controlled release of therapeutic concentrations of CHQ from the transdermal formulation.

To the best of our knowledge, the results of the present study provides the first evidence of the release of therapeautic CHQ concentrations from pectin CHQ matrix patch that cleared the malaria parasites from systemic circulation as well as demonstrating the ability of the transdermal formulation to circumvent the adverse effects of oral CHQ delivery in glucose homeostasis, renal and cardiovascular function markers. This is clinically relevant as it provides a feasible and novel alternative method of CHQ delivery that could play a major role in the effective management of malaria.

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LIST OF ABBREVIATIONS

ACT	artemisinin combination therapy
AIDS	Acquired Immuno Deficiency Syndrome
AVP	arginine vasopressin
cAMP	cyclic adenosine monophosphate
CHQ	chloroquine
CVD	cardiovascular diseases
DA	degree of amidation
DM	degree of methylation
DMSO	dimethyl sulphoxide
GFR	glomerular filtration rate
HDL-c	high density lipoprotein cholesterol
IFN-γ	interferon gamma

IL-10	interleukin 10
LDL-c	low density lipoprotein cholesterol
LPP	lipid protein partitioning
MAP	mean arterial pressure
mmLDL	minimally modified low density lipoprotein
NCC	Na^+-Cl^- cotransporter
NMF	natural moisturizing factors
NO	nitric oxide
oxLDL	oxidized low density lipoprotein
PG	polyethylene glycol
pRBC	parasitized red blood cells
RA	rheumatoid arthritis
RBC	red blood cell
SC-	stratum corneum
SLE	systemic erythematosus
SP	sulphadoxine/pyrimethamine
TB	Tubercolosis
TDD	transdermal drug delivery
TDDs	transdermal drug delivery systems
TNF-α	tumor necrosis factor alpha
VLDL-c	very low density lipoprotein cholesterol
WHO	world health organization

Chapter 1

1.0 Background

Malaria is an acute infectious disease caused by *Plasmodium* parasites and spread by the female anopheles mosquito (WHO 2008, Tuteja 2007). The disease poses a serious global health burden as recent reports have indicated that nearly half of the world's population is at risk (WHO 2008). The World Health Organization (WHO) Expert Consultative Team has further reported that 200-300 million people suffer from acute malaria with a total of 0.5-2.5 million people dying from the disease each year. Sub Saharan Africa is the worst affected by the disease, since 90% of all malaria deaths occur in this region (WHO, 2008). It is noteworthy that despite the numerous global efforts to control and manage the disease through good surveillance and high intervention coverage, malaria remains the third ranked cause of death among the major infectious diseases such as Acquired Immuno Deficiency Syndrome (AIDS) and tuberculosis (TB) (Snow, 2003). Early treatment using effective antimalarial drugs, therefore, remains the major intervention strategy in the control of malaria. However, development of *P. falciparum* resistance to the major, previously effective malaria drugs has hampered global malaria control efforts, demonstrating a need for new therapeutic agents. WHO has recommended use of artemisinin combined therapy (ACT), which have, however, proved to be costly and inaccessible to the majority of people in developing countries (Yeung et al., 2004; WHO, 2006; Whitty et al., 2008; WHO, 2008). Chloroquine (CHQ), therefore, continues to be used in most malaria endemic areas in developing countries despite development of *P. falciparum* resistance to the drug (WHO, 2006). Oral administration is the major delivery route for CHQ. The dosing schedule of 4 tablets at presentation, 2 tablets 6-8 hours later and 2 tablets on the next two consecutive days is, however, relatively inconvenient (Musabayane et al., 2003). Additionally, CHQ's bitterness elicits non-compliance in majority of patients resulting in non completion of the treatment regimen, which has been suggested to be one of the major causes of rapid development of *P. falciparum* resistance to the drug (WHO, 2006). Following the high, initial CHQ doses required for oral delivery, the drug deposits in all epithelial cells of the body to elicit adverse effects in various organ systems (Gustafsson et al., 1983). Current studies have shown that orally delivered CHQ may elicit adverse effects on glucose homeostasis (Smith et al., 1987, Asamoah et al.,

1990), renal (Musabayane et al., 1994, Musabayane et al., 1999) and cardiovascular functions (Sofola et al., 1981, Mubagwa and Adler, 1988, Sofola, 2008). Alternative methods of CHQ administration such as transdermal delivery have, therefore, been suggested, in an effort not only to avoid the bitter taste, but also to modify the dosing schedule, which may improve patient comfort and compliance (Musabayane et al., 2003). Transdermal delivery of CHQ via an amidated matrix patch, which is envisaged to ensure a slow, controlled and sustained release of therapeutic concentrations of CHQ, may circumvent the previously reported adverse effects of oral CHQ. It is against this background that the current study investigated and distinguished between the pathophysiological effects of malaria infection and CHQ treatments on glucose homeostasis, renal and cardiovascular function.

The subsequent sections in this chapter will describe various aspects of malaria disease, control and management using malaria drug armoury. A review of CHQ as an antimalarial drug and associated adverse effects oral CHQ delivery will also be highlighted. Various transdermal penetration enhancers as well as the justification of the study will also be discussed in this chapter.

1.1. Malaria infection

The four identified *Plasmodium* species that cause human malaria are, *Plasmodium vivax, Plasmodium ovale, Plasmodium falciparum, and Plasmodium malariae. Plasmodium falciparum* is the most common in Sub Saharan Africa while *Plasmodium malariae* is sporadic worldwide (Snow, 2003, White et al., 1999, Tuteja, 2007). Occasional infections with monkey malaria parasites, such as *Plasmodium knowlesi* may also occur (Tuteja, 2007). Generally, *Plasmodium berghei*, that only infects rodents, is used for experimental purposes to study human malaria, as it is not hazardous to humans (Janse and Waters, 1995). *P. vivax* and *P. ovale* infect only young red blood cells, *P. malariae* infects only aging cells while *P. falciparum* invades erythrocytes at any age (WHO 2006). The ability of *P. falciparum* to invade erythrocytes of any age explains the heavy parasitaemia generally observed in *P. falciparum* infection. *Plasmodium* infections lead to hemodynamic, immunologic, and metabolic pathogenic features which are initiated by parasitized red blood cells (Barsoum, 2000). Other less common modes of malaria transmission include inoculation of blood from an infected person to a healthy person, such as in blood transfusion (WHO 2003). The *Plasmodium* parasites have a similar, but complex life cycle discussed in the next section.

1.1.1. Life cycle and pathogenesis of *Plasmodium falciparum*

The life cycle of *Plasmodium* occurs in a host (man) and a vector (mosquito). Malaria is acquired through a bite of an infected female *Anopheles* mosquito, which injects the sporozoites into the host's dermis. The sporozoites are then carried in the blood stream to the hepatocytes in the liver. They mature to tissue schizonts, which later releases merozoites into liver sinusoids. This is the extra-erythrocytic cycle (Figure 1).

The erythrocytic cycle starts when the mature tissue schizonts invade the red blood cells (RBC) leading to the formation of ring forms or trophozoites. However, in case of *P. vivax* and *P. ovale*, some sporozoites may go into hibernation, (the *cryptobiotic phase)*, giving rise to *hypnozoites.* The hypnozoites can lie dormant for months or years and upon reactivation they cause clinical relapse (Clark and Schofield, 2000, Mackintosh et al., 2004, Tuteja, 2007). The

trophozoites formed subsequently develop into schizonts that later release a new generation of merozoites. The parasitized red blood cells eventually rupture to release a new generation of merozoites into the blood stream to repeat the same cycle. The sexual cycle in the vector is completed by differentiation of some merozoites into male and female gametocytes (Clark and Schofield, 2000, Mackintosh et al., 2004). The malaria parasite has been shown to have complex metabolic processes such as utilization of amino acids derived from haemoglobin and detoxification of haeme. The enzyme plasmodium aldolase has been identified as one of the enzymes of parasite anaerobic glycolysis (Clark and Cowden, 2003). Reports indicate that the parasites increase the permeability of red blood cell to acquire nutrients, yet maintaining the red blood cell structure intact for at least 48 hours (Clark and Schofield, 2000, Mackintosh et al., 2004). The parasite ingests haemoglobin from RBCs to form a food vacuole where haemoglobin is degraded, releasing haem moeity. The toxic haeme is in turn detoxified by haeme polymerase and sequestrated as haemozoin (malaria pigment). Many of the antimalarial drugs act by inhibiting haeme polymerase thereby causing accumulation of toxic haeme moeity (Clark and Schofield, 2000, Mackintosh et al., 2004). The basic life cycle of all the *Plasmodium* species is similar as shown by that of *P. falciparum* in Figure 1 below.
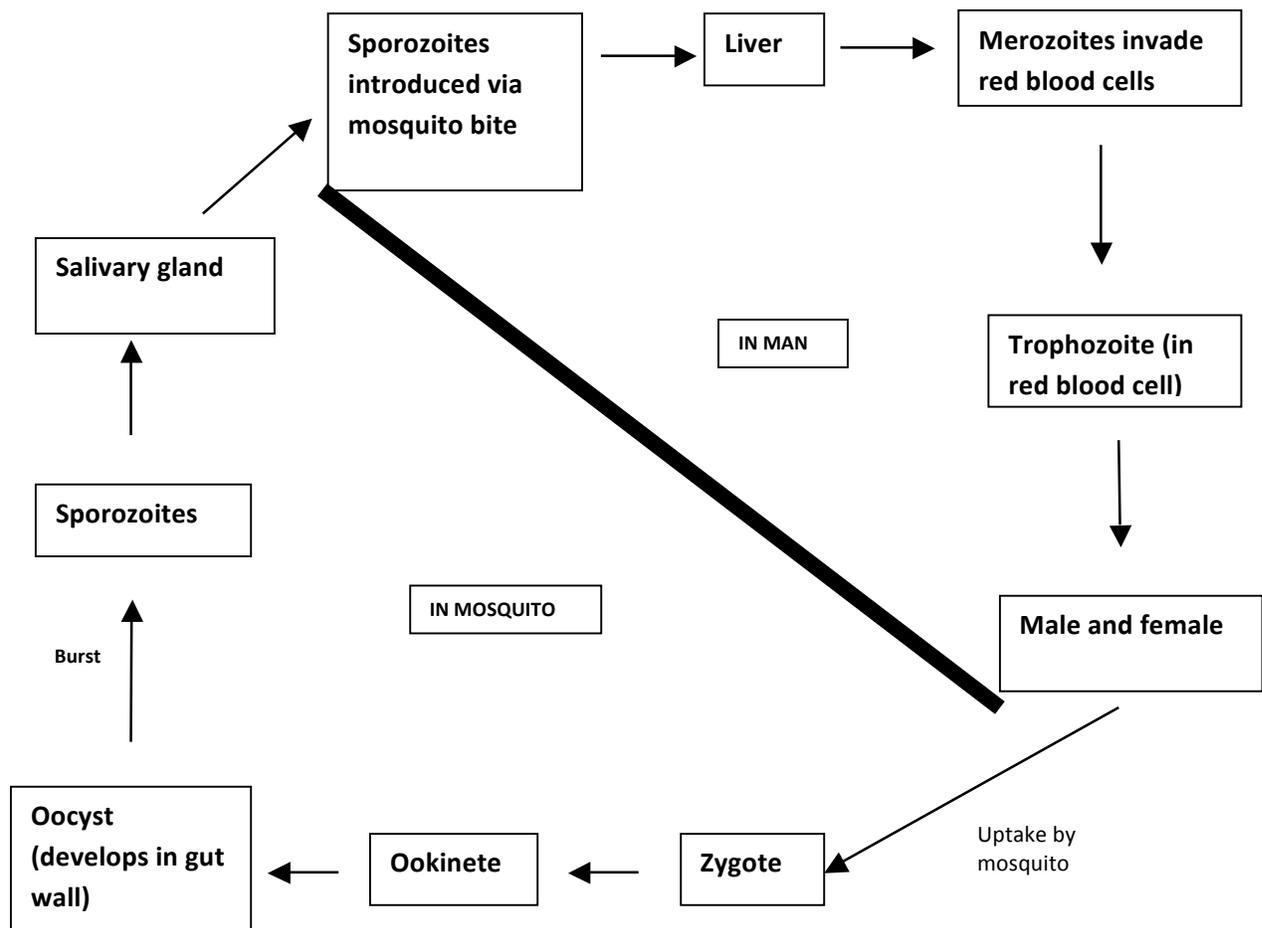
Figure 1: Illustration of the life cycle of malaria parasites of *Plasmodium* spp

The life cycle of the plasmodium parasite is indicative of the general observation that malaria is a complex multisystem disorder, characterized by a wide range of clinical outcomes such as fever and anaemia (Clark and Cowden 2003). The infection of RBCs with malaria parasite is associated with changes in their adhesion properties resulting in RBC deformability which is associated with increased mechanical fragility (Kaul et al., 1991; Kaul et al., 1994; Clark and Cowden 2003). Parasitized RBCs are sticky and thus tend to adhere to adjacent healthy

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erythrocytes, blood platelets, and the capillary endothelium, leading to the formation of intravascular rosettes and clumps that may impede microcirculation (Dondorp et al., 2000, Dondorp et al., 2004). Impediment of microcirculation may lead to hypoxia, reduced metabolite exchange and the release of inflammatory mediators (Clark and Cowden 2003).

A general imbalance in the secretion of pro and anti-inflammatory cytokines in response to malaria induced hypoxia has been reported (Jason et al., 2001; Clark and Cowden, 2003). Elevated plasma concentrations of pro inflammatory cytokines such as TNF-α and IFN-γ and a reduction of anti inflammatory cytokines such as IL-10 may be responsible for pathophysiological and clinical outcomes of malaria. Pro inflammatory cytokines are secreted following release of parasite antigens during the bursting of pRBCs (Clark and Cowden, 2003). Generally, episodic development of fever in malaria infection coincides with episodic bursting of pRBCs to release merozoites during schizogony. However, the fever associated with malaria patients on CHQ treatment is non specific. We speculated that the fever may be caused by the parasite, CHQ or both as literature reports remain unclear to date.

Development of anaemia is another non specific clinical outcome of malaria (Bjorkman, 2002). The pathophysiology of malarial anaemia is relatively multifactorial and complex (Menendez 2000). Current literature evidence has shown that anaemia may result from pathophysiological mechanisms described below. Increased RBC destruction may result from rupture of parasitized red blood cells (pRBC), which occurs in direct proportion to the percentage parasitaemia and schizontaemia (Bjorkman, 2002; Clark and Cowden 2003). Phagocytosis of pRBC also causes a reduction of RBCs in malaria infected patients. The process of phagocytosis is mediated by proliferating and hyperactive macrophages in the reticuloendothelial system (Menendez 2000). Unparasitized RBCs are also prone to phagocytosis as a result of reduced deformability as well as due to the presence of membrane binding parasite antigens. (Menendez 2000; Ghosh and Ghosh, 2007). The spleen also increases the clearance of both parasitized and non infected erythrocytes (Dondorp et al., 1999). Inflammatory processes may cause decreased RBC production due to growth suppression of early precursors of RBC production (Dormer et al., 1983; Menendez 2000; Clark and Cowden 2003). Erythropoietin synthesis has also been

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demonstrated to be suppressed in malaria (Yap and Stevenson, 1994; El Hassan et al., 1997; Dondorp et al., 1999; Chang et al., 2004; Fendel et al., 2010). Dyserythropoiesis has been implicated in decreasing RBC production in *falciparum* malaria as well as eliciting an imbalance of cytokines. (Ghosh and Ghosh, 2007). Excess of tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and nitric oxide (NO) and reduced plasma interleukin (IL-10) have also been associated with excessive RBC destruction in malaria (Menendez 2000; Clark and Cowden 2003; Ghosh and Ghosh, 2007).

It is speculated that the quinoline ring of quinine related antimalarial drugs may act as a hapten combining with specific RBC proteins to become antigenic thereby leading to RBC destruction (Clark and Cowden, 2003). The causes of anaemia associated with malaria patients on CHQ treatment are, therefore, still unclear, indicating that there is need to investigate and distinguish between the pathophysiological effects of malaria and CHQ treatment. Haematocrit measurement is an index of RBC volume blood. Accurate diagnosis of malaria is pivotal in the control of the global burden of malaria as discussed in the next section.

1.1.2. Diagnosis of malaria

Diagnosis of malaria is based on clinical criteria (clinical diagnosis) supplemented by the detection of the parasites in the blood (parasitological or confirmatory diagnosis) (WHO, 2006; WHO 2008). Prompt and accurate diagnosis of malaria is part of effective disease management, whose implementation may effectively help to reduce unnecessary use of antimalarials (WHO, 2006). Confirmatory diagnosis can be done either by the traditional peripheral smear examination using light microscopy and/or rapid diagnostic tests(RDTs) (Rubio et al., 2001, Williams et al., 2008). This improves the specificity of malaria diagnosis thereby reducing drug wastage through unnecessary treatment of patients without parasitaemia. RTDs are more useful in the field when light microscopy is unavailable or during times of an epidemic when diagnosis of all cases using light microscopy is impractical. The use of RTDs is, however, limited by high cost, variable sensitivity and specificity, as well as vulnerability to temperature changes and humidity (WHO, 2006; WHO 2008). WHO, 2008 has previously reported that reduction of

transmission of the malaria parasite is still possible (WHO, 2008). Various ways of controlling malaria transmission are mentioned in the next section.

1.1.3. Control of malaria transmission

Malaria control involves three living beings, man (the host), *Plasmodium* species (the agent), and anopheles mosquito (the vector). Control of malaria transmission may occur through ways that ensure reduced exposure to infected malaria parasites in order to reduce chances of infection (Muturi et al., 2008; WHO 2008). Another level of malaria transmission control involves methods that promote inhibition of vector proliferation and multiplication. Prevention of individual infection requires dedicated and continuous efforts. Every individual living in or visiting malarias areas should adopt personal protection measures against mosquito bites (WHO 2006; WHO 2008). These protective measures include closing of windows and doors, screening of windows, use of mosquito repellant creams, lotions, coils and mats as well as mandatory use of mosquito nets, preferably insecticide treated bed nets. Such travelers should take chemoprophylaxis of anti malarial drugs (WHO 2006; WHO 2008). Mosquito control measures such as source reduction, use of larvicides (chemical, biological) and use of adult insecticides may also be helpful (WHO 2006; WHO 2008). In cases where transmission cannot be avoided altogether, prompt administration of effective antimalarial drugs to infected individuals is another level of malaria transmission control (White, 2008; WHO 2008). The administration of effective treatment is indispensable to averting progression of uncomplicated malaria into severe complicated malaria (WHO 2006; Day and Dondorp, 2007). The major limitation to the control of malaria transmission through administration of effective antimalarial drugs in Sub-Saharan Africa is that medical facilities are limited, poorly equipped and poorly funded, despite being the epicentre of malaria infection (WHO 2006; WHO 2008).

1.2 Drug intervention

Intensification of good surveillance and high intervention coverage in some African countries has made the possible elimination of malaria a realistic goal. The studies done by a WHO Expert Consultative Team reported a decline in malaria cases and deaths by 50%. (WHO, 2008). However, 90% of all the malaria cases and deaths that occur to date due to malaria are in Sub Saharan Africa (WHO 2008). The current global morbidity and mortality due to malaria can be

reduced by access to early and effective treatment (WHO 2006; WHO 2008). Multidrug resistance of the commonly used anti-malarial agents such as CHQ and sulphadoxine/pyrimethamine (SP) combination has been reported in most parts of the world (WHO 2006; 2008). Thus, monotherapy or some of the available combination therapies for malaria are either ineffective or less effective (WHO, 2006). Combination antimalarial drug therapy, the simultaneous use of two or more blood schizontocidal drugs with independent modes of action and different biochemical targets in the parasite, is widely advocated in malaria treatment (WHO, 2006). Treatment of malaria involves administration of a blood schizonticidal and gametocytocidal for preventing relapse and transmission, respectively (WHO 2006). The concept of combination therapy is based on synergistic or additive potential of two or more drugs (Olliaro and Taylor, 2004). Anti-malarial drug combinations can increase efficacy and shorten duration of treatment thereby increasing compliance. This will, therefore, decrease the risk of resistant parasites (WHO, 2006; WHO 2008). The major general limitation of drug combination therapy, however, is the increased cost of the drug. The next section will briefly discuss the currently available antimalarial drug amoury.

1.2.1 Artermisinin derivatives

The medicinal Chinese herb, *Artemisinia annua* is the source of artermisinins, which are currently considered to be the cornerstone of malaria treatment (WHO, 2006). Artemisinin drugs have a rapid onset of action and are considered to be highly potent. The WHO Expert Consultative Group has advocated for the use of artemisinins in combination therapy to reduce development of malaria parasite resistance to the drugs that may occur if they are used as monotherapy (WHO, 2006). The currently available ACTs include artemether-lumefantrine (coartem), artersunate-mefloquine and artesunate-amodiaquine. The mode of action of arteminisins is through the generation of free radicals which cause haemolysis and lysis of infected cells. All the artemisinin derivatives possess both schizontociadal and gametocytocidal activities, giving artermisinin drugs the ability to clear the malaria parasite infection as well as to reduce malaria transmission by reducing gametocyte carriage rates (Nosten and White, 2007, Ogbonna and Uneke, 2008). The major limitations of the drugs include, an imbalance that

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currently exists between demand and supply, comparatively high cost, dosing complexity and basic lack of clinical experience (Bloland, 2003). Literature evidence currently shows that there is no existence of any serious side effects to ACTs in humans. However, toxicity in the nervous system has been reported in animal studies (Greenwood et al., 2008; Kongpatanakul et al., 2009).

1.2.2 Quinine related drugs

The presence of the quinoline ring, characterizes all drugs that are classified as quinine related drugs. Such drugs include quinine, mefloquine, primaquine and chloroquine. Each of these drugs will be discussed briefly, in the next section, except chloroquine, which will be discussed in a later section.

1.2.2.1 Quinine

The shrubs of various species of Rubiacheous genera, Cinchona and Remijia are the sources of quinine, which was the first successful antimalarial drug. Quinine has both schizontocidal and gametocytocidal activities against *P. falciparum, P. vivax and P. malariae (*Barrenes 1996; WHO, 2006). Quinine has, however been shown to be associated with a wide range of side effects collectively known as cinchonism (WHO, 2006). Cinchonism refers to a collection of symptoms such as tinnitus, deafness, nausea, vomiting and vasodilation, which manifests when there is quinine overdose (Bateman et al., 1985). The development of such side effects has generally resulted in patient non compliance.

1.2.2.2 Mefloquine

The drug has schizontocidal activity, but has no effect on the hepatic stage of the malaria parasites (WHO 2006). Mefloquine has a long half life, which has led to contra indications in malaria endemic areas (WHO, 2006). Resistance of *Plasmodium* parasites to mefloquine has been reported, thereby promoting the use of the drug in combination therapy (WHO, 2006; WHO 2008). Winstanley, 1996 reported an association of neuropsychiatric adverse events with mefloquine use (Winstanley, 1996)

1.2.2.3 Primaquine

Malaria relapses that are mainly due to the pre-erythrocytic liver latent forms of *P. vivax and P ovale* can be eliminated using primaquine, an 8 aminoquinoline drug (Jeans and Heard, 1999, WHO 2006). Primaquine possesses both blood and tissue schizontocidal and gametocytocidal activities (Butcher, 1997; WHO 2006; WHO 2008). The drug's major limitation is the precipitation of haemolytic anaemia in glucose-6-phosphate dehydrogenase (G-6-P) deficient patients (Jeans and Heard, 1999).

1.2.2.4 Chloroquine

CHQ, a diprotic compound possesses both blood schizonticidal and gametocytocidal activities against CHQ sensitive *Plasmodium* parasites (Butcher, 1997; WHO, 2006). CHQ mode of action and effects on various organ systems will be discussed in a later section.

1.2.3. Antifolates

Inhibition of folate metabolism of *P. falciparum* is the target site for antifolate drugs such as pyrimethamine, sulphadoxine and proguanil (Bzik et al., 1987). Antifolate drugs inhibit key enzymes, dehydrofolate reductase (DHFR) and dehydropteroate synthase (DHPS) in the parasite's folate metabolic pathway (REF). Interference with these enzymes compromises the survival of the malaria parasite. Antifolates, targeting different enzymes in the folate patway have been used in combination therapy, such as the pyrimethamine/sulphadoxine combination. The major limitation of the drug combination is the development of *P. falciparum* resistance to the drug (Nwanyanwu et al., 1996). Pyrimethamine interferes with DHFR while sulphadoxine has been shown to interfere with DHPS enzyme activities, respectively. Proguanil, which is used in malaria prophylaxis, reportedly inhibits the DHFR as the drug's mode of action (Mulenga et al., 2006). However, further development of proguanil has been hampered by development of *P. falciparum* resistance to the drug (Mulenga et al., 2006).

1.2.4 New antimalarial drugs

Due to the escalating costs of new drug developments, the modifications in the structure of existing antimalarial drugs has been the common focus in an effort to introduce new antimalarial drugs. Another strategy may be the reformulation of existing drugs. Combination of existing antimalarial drugs may also be useful (Fivelman et al., 1999; Biagini et al., 2003; Kremsner and Krishna, 2004; Lederman et al., 2006; WHO, 2006; Deen et al., 2008; WHO, 2008). These strategies are exemplified by the artemether/lumefantrine combination as well as dihydroartemisinin/piperaquine combination (WHO, 2006; WHO, 2008). In each case, a fast acting blood schizonticidal drug with gamecytocidal activities is combined with a slow acting drug that will clear the parasites slowly in order to avoid recrudescence (WHO, 2006; WHO, 2008)

1.2.5 Optimisation of therapy

Amtimalarial drug therapy may be optimised by the use of analogues of existing traditional antimalarial agents. The strategy has been very successful in quinine related drugs, where it led to the introduction of CHQ, primaquine and mefloquine based on the chemical modification of quinine (Winstanley, 2000; WHO, 2006). Antimalarial drug therapy may also be optimized by using the traditional, currently available antimalaria agents as drug combinations. One such combination is amodiaquine/ sulphadoxine/pyrimethamine combination (WHO, 2006). The use of natural plant products may also be an important adjunct in the treatment of malaria. Indeed many successful drugs to treat various ailments have been extracted from plants. There are, therefore, many plant based products that are currently being evaluated as potential antimalarial treatment options. However, they will not be reviewed in this study, as they are outside our scope.

As previously mentioned, CHQ continues to be a drug of choice in Sub Saharan Africa due to its wide availability and accessibility despite reported development of parasite resistance to this drug. As such, the next section comprehensively discusses the structure, mode of action and delivery routes that are employed for this drug.

1 3 Chloroquine

CHQ continues to be the major chemotherapeautic agent used in the treatment and prophylaxis of malaria in most malaria endemic tropical countries (WHO 2006; WHO 2008). This is despite the worldwide emergence of *falciparun* resistance to CHQ treatment which has forced the majority of African countries to deploy other available drugs as their first line of therapy (White et al., 1999, Molyneux et al., 1999, Baird, 2000). Various factors such as inadequate dosing, incomplete courses of therapy, indiscriminate and inappropriate use, and reliance on less effective medications were the major contributors to the development of *falciparum* resistance to CHQ (Karbwang and Harinasuta, 1992, Bloland et al., 1993). However, in Sub Saharan Africa, the alternative, ACT based drug combinations are generally inaccessible, hence the continued use of CHQ (WHO 2008). CHQ's affordability, easy administration, wide availability and rapid onset of action has potentiated the drug's continued use (WHO 2006; WHO 2008). Current literature evidence indicates that CHQ is not only used to treat malaria, but other disease conditions such as systemic erythomatosus (SLE) and rheumatoid arthritis (RA). The lysosomotropic properties of CHQ have enabled the drug to assume such a plethora of uses.

1 3.1 Absorption, metabolism and excretion

Following oral administration, maximum plasma concentrations of CHQ are attained after 1-4 hours, which is indicative of the drug's rapid absorption (Adelusi et al., 1982; Gustafsson et al., 1983, Walker et al., 1983, White, 1992; Augustijns and Verbeke, 1993, Vries et al., 1994). CHQ has the ability to bind avidly to different epithelial cells and body tissues, attributed to the drug's large volume of distribution in the body. The drug has a long elimination half life which varies between plasma and tissues (McChesney et al., 1967, Adelusi and Salako, 1982, Wetstein et al., 1995; Ahmed et al., 2003a). Gene mutations may occur in the major CHQ transporter, PfCRT, results in reduced CHQ uptake by RBCs in CHQ resistant strains thereby limiting the effectiveness of the drug (Warhurst, 2001). About 70% of CHQ ingested dose is excreted unchanged in the urine (Pussard and Verdier, 1994). Current literature evidence has shown that the cytochrome P- 450 (CYP) isoform enzymes are involved in CHQ metabolism in humans (Projean et al., 2003)

1.3.2 Mode of action

The mechanism of action of CHQ is still not completely understood. (Raynes, 1999). Yayon and Ginsburg, 1983 postulated that CHQ may interfere with the function of the food vacuoles in the mature stages of the erythrocytic parasite (Yayon and Ginsburg, 1983). Based on the physico chemical properties, CHQ rapidly accumulates within the acidic food vacuole of the parasites to exert therapeutic effects (Yayon et al., 1985; Fitch and Russell, 2006). The food vacuole is the site of haemoglobin breakdown, to amino acids that nourish the parasite. The by-products of haemoglobin breakdown are the toxic haem moieties, also referred to as ferriptoroporphyrin IX (FP). FP has been shown to be toxic to RBC membranes and the malaria parasite (Slater et al., 1991, Slater and Cerami, 1992; Sullivan et al., 1996; Bray et al., 1998; Bray et al., 1999; Fitch and Russell, 2006). However, the parasite has the ability to detoxify haematin into non toxic, insoluble and inert haemozoin through unclear mechanisms. CHQ exerts antimalarial activity by interfering with the process of haem detoxification (Fitch and Russell, 2006). The drug binds with a high affinity to haematin, inhibiting its sequestration into haemozoin (Slater, 1993; Egan et al., 1994; Francis et al., 1997; Bray et al., 1998; Foley and Tilley, 1998; Bray et al., 1999). The result is the generation of toxic CHQ-haem complexes as well as accumulation of free haem moieties within the parasite's food vacuole (Pandey et al., 2001). The accumulation of such moieties is associated with RBC lyses and depressed activity of key enzymes the parasite requires for metabolic pathways.

Based on the pharmacokinetic properties of CHQ and the drug's ability to bind to all epithelial cells, we speculated that the initial high oral doses of CHQ could be toxic to organ systems. On the other hand, the pathogenesis of malaria may also exert pathophysiological effects to organ systems. Currently, there is no literature evidence that distinguishes between the pathophysiological effects of malaria and CHQ in malaria patients. The next section discusses the possible pathophysiological effects of malaria and CHQ on glucose homeostasis, renal and cardiovascular function.

1.4 Impaired glucose homeostasis

The pathogenesis and metabolic perturbations of malaria show that the malaria parasite and associated fever influences glucose homeostasis of the human host. The parasite heavily and completely depends on the host for all its energy requirements, which impact negatively on the host's energy stores (Phillips 1989). The overall effect may be an imbalance between the host's energy demands and supply which may precipitate hypoglycaemia in patients with glucose-insulin mismatching (Phillips 1989). On the other hand, CHQ has also been shown to affect glucose homeostasis through its effects on insulin secretion and breakdown of the activated hormone (Knutson, Ronnett and Lane, 1985; Phillips 1986; Davis, 1997; Jarzyna, Kiersztan, Lisowa and Brya, 2001). We speculated that, such effects on blood glucose and plasma insulin concentrations could affect glycogen stores, in the muscle and liver of the host cell. The following section will discuss the independent effects of malaria and CHQ on glucose homeostasis.

1.4.1 Effects of malaria on glucose homeostasis

Consumption of host glucose to meet the parasite's energy demands may precipitate hypoglycaemia through varied mechanisms. One such proposed mechanism is through depletion of vital gluconeogenic substrates such as thiamine (Krishna et al., 1999). Thiamine depletion is usually preceded by increased serum transketolase activity in patients with *falciparum* malaria in response to increased demand for parasite's glycolytic pathway (Krishna et al., 1999). Thiamine depletion has also been associated with a depression of the host's aerobic glycolysis, resulting in increased anaerobic glycolysis and subsequent lactic acid accumulation (Krishna et al., 1999).

In addition to malaria induced depletion of gluconeogenic substrates, liver damage associated with malaria may further precipitate hypoglycaemia. The rapid proliferation and migration of malaria parasites from one hepatocyte to another during the life cycle of the malaria parasite has been shown to cause hepatocellular damage (Dekker et al., 1997). The possible leakage of the intracellular contents of the damaged hepatocytes to the extracellular fluid may occur, as evidenced by studies that have reported elevated plasma concentrations of liver enzymes such as

aspartate and alanine transaminases as well as alkaline phosphatase to be indicative of such liver damage (Kausar et al., 2010). The liver is key organ involved in the maintenance glucose homeostasis; hence any hepatocellular damage may impair glucose homeostasis. The liver plays a major role in breaking down the activated insulin-receptor complex. Hepatocellular damage may, therefore, result in slow insulin receptor recycling, thereby precipitating hypoglycaemia (Onyesom and Agho, 2011).

1.4.2 Effects of CHQ on glucose homeostasis

The effects of CHQ on glucose homeostasis remain obscure to date. Current literature evidence generated from both human and animal experimental studies have reported conflicting findings on the effects of CHQ on blood glucose concentration. Smith et al, 1987, showed that CHQ has no effect on plasma glucose concentration in non diabetic subjects (Smith et al., 1987) while a separate study using experimental animals and high doses of CHQ, reported that CHQ causes hyperglycaemia (Obi et al., 2003). This conflicting evidence is indicative that CHQ induced effects on glucose homeostasis remain unclear.

Davis, 1997, has reported d that CHQ reduced basal plasma glucose levels and increased basal plasma insulin levels, possibly mediated through decreased insulin clearance and increased peripheral uptake of glucose (Davis, 1997). However, a short term study which lasted for 4 weeks, gave different results. These investigators reported decreased blood glucose concentrations during the first week of study which returned to baseline levels thereafter for the duration of the study (Jimmy et al., 2007). Jarzyna et al, 2001, reported increased peripheral glucose disposal and reduced insulin resistance in insulin-dependent diabetes mellitus in experimental animals (Jarzyna et al., 2001).

Numerous studies, therefore, seem to suggest that CHQ may induce hypoglycaemia through varied and unclear mechanisms (Knutson, Ronnett and Lane, 1985; Davis, 1997; Jarzyna, Kiersztan, Lisowa and Bryla, 2001). The suggested CHQ induced hypoglycaemia has been ascribed to decreased insulin degradation (Knutson, Ronnett and Lane, 1985) or increased insulin secretion. (Davis, 1997). Bevan et al, 1995, has reported that hypoglycaemia may be due to CHQ

induced extension of the life span of CHQ-insulin activated complex (Bevan et al., 1995). CHQ, being a diprotic base, rapidly accumulates in acidic lysosomes and endosomes thereby elevating lysosomal pH. An elevation of lysosomal pH compromises the optimal activity of lysosomal hydrolases that degrade hepatic insulin, with a concomitant increase in plasma concentration of intact insulin (Knuston, Ronnet and Lane, 1985). Smith et al, 1987, has also reported that non insulin dependent diabetic patients that were given CHQ, had increased plasma insulin concentration with a concomitant improvement in their glucose tolerance (Smith et al., 1987). CHQ has also been shown to increase insulin secretion via rapid accumulation in the pancreas, thereby having a direct effect on the beta cells of islets of Langerhans (Asamoah et al., 1990).

CHQ induced hypoglycaemia has also been ascribed to inhibition of gluconeogenesis due to CHQ induced hepatocellular damage (Jarzyna, Kiersztan, Lisowa and Bryla, 2001). CHQ induced hepatocellular damage may lead to inhibition of key gluconeogenic enzymes; glutamate dehydrogenase and glucose-6-phosphatase (Jarzyna et al., 2001). It may also lead to decreased transport of pyruvate into hepatocyte mitochondria (Jarzyna et al., 2001) with a concomitant decrease of gluconeogenesis, which may eventually cause hypoglycaemia.

In summary, administration of oral CHQ in a malaria patient could possibly have a major impact on the patient's glucose homeostasis as evidenced by the fact that both the disease itself and the drug may be having independent effects on this system. However, as will be discussed in later sections, alternative and novel CHQ delivery methods such as transdermal delivery could avert the aforementioned adverse effects of oral CHQ on blood glucose homeostasis. This is due to the controlled and sustained release of CHQ from this formulation, thereby averting the challenges of dose dumping associated with oral CHQ delivery.

As such, this study sought to investigate and distinguish between pathophysiological effects of malaria and CHQ on blood glucose and plasma insulin concentration, in experimental animal paradigms.

1.5 Adverse renal effects

Falciparum malaria is a complex disease with a broad clinical spectrum, involving several pathophysiological changes which include, capillary endothelial damage, increased vascular permeability, impaired renal microcirculation, haemodynamic alterations, haematologic changes, and immunologic responses(Boonpucknavig and Sitprija, 1979; Weller et al., 1992; English et al., 1996; Prakash et al., 1996; Basoum, 2000; Prakash et al., 2002; Naqvi et al., 2003; Das, 2008; Ogbadoyi and Tsado, 2009; Idonije et al., 2011). Renal involvement in malaria varies widely due to the complexity of the disease (Boonpucknavig and Sitprija, 1979; Basoum, 2000). On the other hand, impairment of renal function has also been independently ascribed to CHQ treatment. This is, therefore, indicative of the need to distinguish between the pathophysiological effects of malaria and chloroquine on renal function.

1.5.1 Malaria induced adverse renal effects

The specific effects of acute *falciparum* malaria on renal impairment remain obscure to date (Newton et al., 1991, Newton et al., 1994). There are various complicated pathophysiological mechanisms in the pathogenesis of malaria that have been shown to be responsible for the reported effects of malaria on renal function (Barsoum, 2000). There are two major renal syndromes associated with malaria, namely, a chronic and progressive glomerulopathy and acute renal failure (Boonpucknavig and Sitprija, 1979; Barsoum, 2000). Renal disturbances in acute renal failure may be attributed to impaired renal microcirculation. (Boonpucknavig and Sitprija, 1979; Prakash et al., 1996; Barsoum, 2000; Prakash et al., 2002; Ekeanyanwu and Ogu, 2010)

Malaria cute renal failure has also been attributed to haemoglobinuria caused by massive intravascular haemolysis and glomerular lesions as a result of immunologic reaction to parasites (Boonpucknavig and Sitprija, 1979; Barsoum, 2000). The non specific effects of fever have also been shown to be responsible for previously reported fluid and electrolyte disorders in malaria acute renal failure (Barsoum, 2000).

Malaria acute renal failure is associated with varying degrees of fluid and electrolyte imbalances characterized by extracellular fluid volume depletion, azotaemia and hyponatraemia (Boonpucknavig and Sitprija, 1979; Prakash et al., 1996; Das, 2008).

Suggested mechanisms involved for malaria induced hyponatraemia include increased plasma concentration of AVP characterized by hypervolemia, high urine sodium concentration and hyperosmotic urine (Prakash et al., 1996; Elsheikha and Sheashaa, 2007, Ehrich and Eke, 2007, Das, 2008). Renal ischaemia and tubular obstruction by haemoglobin casts byproducts of RBC haemolysis in malaria, have also been shown to be involved in the development of malaria acute renal failure. In the early stages of acute malaria renal failure, constriction of the renal vascular bed and splachnic blood vessels occurs (Boonpucknavig and Sitprija, 1979; Das, 2008) leading to renal ischaemia. The resultant renal ischaemia causes a decrease in urine volume, urinary sodium excretion, increased urine osmolality, and decreased GFR mediated via malaria induced increases in the sympathetic activity (Elsheikha and Sheashaa, 2007, Ehrich and Eke, 2007, Das, 2008). Acute malaria renal failure is also associated with blood hyperviscosity resulting from decreased deformability of parasitized erythrocytes which leads to decreased blood flow in renal microcirculation (Elsheikha and Sheashaa, 2007, Ehrich and Eke, 2007, Das, 2008).

1.5.2 CHQ induced adverse renal effects

Acute administration of CHQ has been shown to increase urine flow rate and protein excretion (Ngaha, 1982). We have previously shown that acute administration of CHQ causes natriuresis, without affecting urine output (Musabayane et al., 1993, Musabayane et al., 1996). We have also reported CHQ stimulated AVP secretion following acute CHQ administration (Musabayane et al., 2000c). CHQ reportedly suppressed AVP induced cyclic adenosine monophosphate (cAMP) production at the level of the hormone receptor complex (Musabayane et al., 2000c). Our previously reported absence of antidiuresis despite CHQ induced increases in plasma AVP concentration may be explained by this mechanism. (Musabayane et al., 1996; Musabayane et al., 2000c). CHQ antagonises AVP induced cAMP generation in the inner medullary collecting ducts. The result is the inability of AVP to increase the concentration of intracellular cAMP, thereby inhibiting the antidiuretic action of the hormone (Musabayane et al., 2000c)..

Additionally, we have shown that AVP-induced increases in renal Na^+ excretion are mediated via V_1 receptors (Musabayane et al., 2000b).

On the other hand, CHQ induced NO has inhibitory effects on cAMP production with an overall effect of making the collecting duct unresponsive to the action of vasopressin. However, Ahmed et al, 2003 have attributed CHQ's diuretic, natriuretic effects and its ability to increase both GFR and plasma vasopressin to its ability to stimulate NO secretion. The ability of CHQ to directly inhibit cAMP generation while stimulating NO production, accounts for its overall modulation of renal tubular response (Ahmed Ashton & Balment, 2003a). Overall, Ahmed et al., 2003, attributes the effects of CHQ and vasopressin secretion to be a nitric oxide dependent effect (Ahmed Ashton & Balment, 2003a). Chronic administration of CHQ, has been reported to, cause Na^+ and Cl^- retention and reduction in both GFR and blood pressure. Sodium retention is expected to increase extracellular fluid volume and subsequently increase MAP (Musabayane et al., 1994). Surprisingly, MAP was reportedly reduced due to extensive vasodilatation that occurs following CHQ administration, (Ahmed Ashton & Balment, 2003a).

1.6 Adverse cardiovascular effects

Malaria induced cardiovascular impairment has been generally associated with metabolic disturbances attributable to abnormalities in the host's RBC membranes, the parasite's consumption of nutrients and cofactors, haemodynamic and immunologic disturbances. CHQ has also been shown to have adverse cardiovascular effects (Sofola et al., 1981, Mubagwa and Adler, 1988, Sofola, 2008). Therefore, there is need to distinguish between the pathophysiological effects of malaria and CHQ, in order to comprehend the cardiovascular complications in malaria patients.

1.6.1 Malaria induced adverse cardiovascular effects

P. falciparum infection is generally associated with cytoadherence and sequestration of pRBCs and unparasistised RBCs within coronary microcirculation microcirculation (Dondorp et al., 2000, Dondorp et al., 2004). The blood vessels are small, resulting in microvascular pathology and obstruction to coronary blood flow. Infected RBCs tend to stick to uninfected RBCs, forming rosettes, which may clog the microcirculation microcirculation (Dondorp et al., 2000, Dondorp et al., 2004). The overall result is tissue hypoxia, which may lead to anaerobic respiration, lactic acid production and eventually generation of reactive oxygen species (Clark and Cowden 2003).Uyemura et al., 2000 have previously suggested that malaria induced lactic acid production may result in depressed mitochondrial respiration (Uyemura et al., 2000). The overall result is increased lipid peroxidation and oxidative stress (Arun-Kumar and Das, 1999). We speculated that an increase in oxidative stress may alter the lipid profile status and blood pressure of malaria infected animals.

1.6.2 CHQ induced adverse cardiovascular effects

CHQ has been shown to be associated with cardiac nerve impulse conduction disturbances (Sanguinetti and Jurkiewicz, 1990, Shah, 2005, Owens and Nolin, 2006). Literature evidence has demonstrated that CHQ may delay ventricular depolarization resulting in widening of the QRS complex, particularly the QT prolongation (Sanguinetti and Jurkiewicz, 1990, Shah, 2005, Owens and Nolin, 2006). The ability of CHQ to cause conduction disturbances has also been demonstrated by Mubagwa and Adler, 1988, who reported that CHQ increased heart rate in a dose dependant manner. They suggested the effects to be mediated via muscarinic receptors (Mubagwa and Adler, 1988). Sofola, 2008, also reported that CHQ prolonged the PR interval on the ECG and caused bradycardia in a dose dependent manner, suggesting that beta-adrenergic blockade renders the heart more vulnerable to the actions of CHQ (Sofola et al., 1981, Sofola, 2008). However, following intravenous administration, only prolongation of QRS interval has been reported (Bustos et al., 1994). The ability of CHQ to cause conduction disturbances is further shown by studies that have demonstrated that at low micromolar concentrations, CHQ

lengthened the action potentials of cat Purkinje fibres, and increased automaticity (Benavides-Haro and Sanchez-Chapula, 2000, Sanchez-Chapula et al., 2001).

CHQ has also been shown to cause hypotension (Looareesuwan et al., 1986; Marquardt and Albertson, 2001; Messant et al., 2004). CHQ induced hypotension was observed during CHQ self- poisoning, where there was both tachycardia and bradycardia with atrioventricular block. In such cases, intraventricular conduction delay as indicated by widening of the QRS interval has also been consistently reported. Such patients present with shock resulting from CHQ's ability to cause peripheral vasodilatation and negative inotropic effect (Riou et al., 1988, Riou et al., 1989). Hypotension associated with CHQ is a result of arteriolar dilatation and venodilatation. Studies by Abiose et al. demonstrated CHQ induced venodilation via the release of NO in human forearm veins (Abiose et al, 1997). The results of this study, further demonstrated that CHQ may reduce both preload and afterload when oral dosages above the recommended therapeutic doses are taken. Other mechanisms that have been suggested to explain the previously reported CHQ induced hypotensive effects, were CHQ ability to cause blockade of alpha receptors and increased histamine secretion (Abiose et al, 1997). However, to date, information as to other cardiovascular effects of CHQ in malaria setting are still unclear. We therefore, speculated that CHQ may affect the plasma lipid profile parameters, which is a more sensitive marker of CVD impairment.

Generally, lipid metabolism is a complex but highly regulated physiological process (Kwiterovich, 2000). Dyslipidaemia, which usually leads to development of atherosclerosis, may include both lipoprotein overproduction and deficiency manifesting in any one or more of the following; elevated total cholesterol, low-density lipoprotein cholesterol (LDL-c), and triglyceride levels or as decreased high-density lipoprotein cholesterol (HD-c) level (Grundy, 1995; Lamarche et al., 1999). The pro atherogenic particles, therefore, include LDL-c, very low density lipoprotein (VLDL) and triglycerides, while HDL-c has a protective effect through multiple mechanisms (Grundy, 1995; Libby, 2001; Toth, 2005; Natarajan et al., 2010). HDL-c transports cholesterol from peripheral tissues to the liver (Navab et al., 1996, Ross, 1999; Jin et al., 2002; Alsheikh-Ali et al., 2005).

Initiation of atherosclerosis occurs via development of a fatty streak which is induced by oxidation of LDL in the sub endothelial space (Navab et al., 1996; Holvoet and Collen, 1998; Libby et al., 2000). LDL progresses from being minimally modified (mmLDL) to being extensively modified (oxLDL) (Schwenke and Carew, 1989; Navab et al., 1996). The mmLDL and inflammatory cytokines promote the secretion of adhesion molecules that induce adhesion of monocytes to the endothelial cells. The activated monocytes then migrate into the sub endothelial space where they differentiate into macrophages. The result is the formation of foam cells via uptake of oxLDL by scavenger receptors located on the surface of macrophages (Ross, 1999; Libby et al., 2000). The oxLDL taken up by macrophages are then esterified, stored in lipid droplets and then converted into soluble forms or exported to extracellular HDL through the action of cholesterol transporters that include ABC-A1. Atherosclerotic lesion progression is a result of interactions between macrophage foam cells, Th1 and Th2 cells establish a chronic inflammatory process (Ross, 1999). All the cellular components in the vessel wall are then subjected to both pro- and anti atherogenic actions of cytokines secreted by lymphocytes and macrophages. There is also migration and proliferation of smooth muscle cells from the media to the arterial intima, where they form a fibrous plaque via the secretion of extracellular matrix proteins (Ross 1999; Esteve et al., 2005). Plaque rupture and thrombosis results from necrosis of foam cells derived from macrophages and smooth muscle cells (Ross, 1999; Gau and Wright, 2006). This leads to the formation of a necrotic core characterized by accumulation of extracellular cholesterol. Weakening of the fibrous plague occurs as a result of macrophage secretion of matrix metallo-proteinases and neo-vascularization. This leads to plague rapture exposing blood components to tissue factor thereby initiating coagulation, recruitment of platelets, and subsequent formation of a thrombus (Ross, 1999; Esteve et al., 2005; Gau and Wright, 2006). Atherosclerosis may, therefore, eventually lead to cardiovascular complications, such as myocardial infarction and stroke.

The effects of CHQ on the plasma lipid profile parameters have remained unclear. Indeed, studies on systemic erythematosus (SLE) and rheumatoid arthritis (RA) patients have given obscure findings. None of the studies in literature have investigated the effects of CHQ on the

lipid profile parameters, in a malaria model. Beynen, 1986 reported that CHQ and its analogues, quinacrine and hydroxychloroquine have the ability to treat hypercholesterolaemia (Beynen, 1986). In SLE patients, CHQ was shown to lower total cholesterol (Wallace et al., 1990, Hodis et al., 1993, Petri et al., 1994, Kavanaugh et al., 1997, Rahman et al., 1999, Tam et al., 2000b, Tam et al., 2000a). The suggested mechanism for this reduction was via reduction in hepatic cholesterol synthesis and preferential reduction in the synthesis of VLDL. In some of the above mentioned studies, CHQ and other antimalarial drugs were also able to reduce plasma VLDL (Hodis et al., 1993, Tam et al., 2000a). However, reports by Beynen et al, 1986, have also shown that CHQ may have beneficial effects by reducing levels of pro atherogenic lipoproteins such as LDL-c (Wallace et al., 1990). Some studies have also shown ability of CHQ to increase HDL in patients with rheumatoid arthritis (Munro et al., 1997) as well as in SLE patients (Borba and Bonfa, 2001). HDL has cardioprotective properties against development of atherosclerosis. It must be noted, however, that the pathogenesis of SLE or RA are different from malaria, hence the effects of CHQ on the plasma lipid profile parameters in malaria patients may vary.

1.7 Novel chloroquine delivery methods

Much interest has arisen leading to a continued search for long-acting CHQ preparations to modify the dosing schedule (Musabayane et al., 2003). Novel CHQ formulations that allow the bitter taste to be masked or avoided provide attractive alternatives (Musabayane et al., 2003). One such alternative drug route, transdermal delivery would avert adverse effects discussed above attributable to transiently high plasma CHQ concentrations following oral administration.

1.7.1 Transdermal delivery

Transdermal delivery refers to delivery of a drug into the systemic circulation via the skin (Foldvari, 2000, Benson, 2005, Morrow et al., 2007, Prausnitz and Langer, 2008). This method of delivery is an attractive approach for either local or systemic treatment in medicine (Cevc et al., 1996). Current literature evidence has shown that the skin is becoming a new frontier for the delivery of a wide variety of drugs, thereby avoiding inconvenient, repetitive dosing schedules associated with conventional oral delivery and use of hazardous hypodermic needles (Foldvari, 2000). The ultimate goal of transdermal drug delivery is to ensure that compounds are delivered, preferably at a sustained, controlled specific rate, to the systemic circulation (Morrow et al., 2007, Songkro, 2009). There are numerous advantages which make transdermal delivery the first choice drug delivery route over oral delivery (Prausnitz and Langer, 2008). However, the stratum corneum (SC) layer of the skin provides a barrier function, which is the major impediment to effective utilization of this delivery route to deliver various drugs. As such, it is imperative to discuss the physiology of the skin, possible routes of drug penetration, advantages and limitations of this drug delivery route as well as various techniques and methods that can be used to enhance drug permeation through the skin.

1.7.2 Physiology of the human skin

To understand drug delivery through the skin one should first become familiar with the structure of the skin barrier (Barry, 2001). An understanding of the structure and barrier properties of the SC is indispensable in the quest to optimize transdermal drug delivery. The skin, the largest organ of the body is comprises of several layers which include the uppermost layer referred to as SC, the epidermis, the dermis, and the lower layers of adipose tissue (Powell and Soon, 2002,

Powell, 2006, Venus et al., 2010). Human skin is an effective, selective barrier to chemical permeation since the primary role of the SC is to provide a substantial diffusional barrier and thereby protect the body from by xenobiotics (Powell and Soon, 2002, Powell, 2006, Venus et al., 2010). Biophysical, morphological, and biochemical evidence indicates that the SC forms a continuous sheath of protein-enriched corneocytes embedded in an intercellular matrix enriched in non-polar lipids and organized as lamellar lipid layers (Bouwstra and Honeywell-Nguyen, 2002). SC is, therefore made up of 10-15 layers of corneocytes whose thickness at any given time depends on its state of hydration (Bouwstra and Honeywell-Nguyen, 2002, Menon, 2002). The SC layer comprises keratin-rich corneocytes (brick) and an intercellular matrix (mortar) composed of long chain ceramides, free fatty acids, triglycerides, cholesterol, cholesterol sulphate and sterol/wax esters. This molecular arrangement gives a multi- layered 'brick and mortar' like elegant structure (Powell and Soon, 2002, Powell, 2006, Venus et al., 2010). The intercellular lipid matrix is generated by keratinocytes giving a lamellar structure (Powell and Soon, 2002, Powell, 2006, Venus et al., 2010). These multiple, lipid bilayers tend to form regions of semi crystalline gel and liquid crystals domain. Therefore, this uniform, ordered nature of these biochemicals, especially lipid bilayers maintain and promote the diffusional resistance of the SC barrier. Directly below the stratum corneum is the viable epidermis, which consists of three layers, the stratum granulosum, spinosum and basale. The epidermis contains keratinocytes at varying stages of differentiation, as well as melanocytes, Langerhans cells which are important for antigen presentation and immune response, and Merkel cells which are involved in sensory perception (Cevc et al., 1996, Venus et al., 2010). Epidermis is the region where the transdermally delivered drug can either be absorbed into circulation, metabolized or if it is a prodrug, may be activated. In this way, the drug is cleared rapidly from the skin tissues (Goodman and Barry, 1989). Underneath the epidermis is the dermis, which consists of fibroblasts, endothelial cells and mast cells. These cells are embedded in connective tissue composed of mainly collagenous fibres, and this accounts for approximately 70% of the dry weight of the skin (Rivierea and Papich, 2001, Trommer and Neubert, 2006)

The SC, therefore, poses a formidable challenge to the formulation of transdermal drug delivery systems (Trommer and Neubert, 2006). However, the presence of intrinsic and extrinsic

proteins, such as enzymes, may affect the lamellar structure (Venus et al., 2010). One very vital component of the SC is water which acts as a plasticizer to prevent cracking and in maintaining suppleness by generating a natural moisturizing factor (Menon, 2002, Powell, 2006). This state of hydration is vital to enable enhanced drug permeation. Several approaches have been utilized to facilitate entry of drugs into the lower skin layers, and subsequently into systemic circulation (Barry, 2001). There are various routes that a drug can use to penetrate the skin although the transport across the continuous SC is the most important.

1.7.3 Drug transport through the human skin

There are three potential pathways with which drugs can penetrate the SC, viz: through the sweat ducts, via the hair follicles and sebaceous glands (shunt or appendageal route), or directly across the SC (Potts and Francoeur, 1990, Potts and Guy, 1992, Songkro, 2009). The bulk of skin penetration enhancement techniques continues to focus on drug transport across the continuous SC rather than via the appendages as their contribution to a steady state flux of drugs is reportedly very minimal (Barrett, 1969, Foldvari, 2000, Barry, 2001).

1.7.3.1 Routes of drug penetration through the SC

Drugs can penetrate the SC using either the micro or macro routes (Barrett, 1969). Micro routes include intercellular and transcellular delivery routes, whereby the specific pathways followed by the drug may be between adjacent cells or traversing through the cells. Most drug penetration enhancers tend to disrupt the ordered SC structure in order to promote both intercellular and transcellular transport systems (Songkro, 2009). Macro routes of drug penetration refer to transport at a large or bulk scale across the SC. The macro routes include transport via the sweat ducts and hair follicles which make up the skin appendages, collectively referred to as the shunt transport system. This shunt system is generally important for the transport of ions, large polar molecules, polymers and colloidal particles(Barry, 2001, Songkro, 2009). Another vital transport pathway is directly across the continuous SC, which forms the major route of transdermal drug penetration. In this penetration pathway, each drug has to undergo a series of partitioning into and diffusing across multiple hydrophilic (keratinocyte) and hydrophobic (lipid lamellae) domains. This is reportedly a major limitation to transdermal delivery of most drugs, as the

permeation pathway is dependant not only on the physicochemical properties of the drug, but of the vehicle as well (Trommer and Neubert, 2006). Generally drug permeation across the SC obeys Fick's first law, which is dependent on solubility and partition coefficient of a drug (Barry, 2001, Songkro, 2009). Transdermal delivery is highly feasible for drugs with a low relative molecular mass, hence for most drugs, there is need to manipulate the physicochemical properties of both the drug and its vehicle. Transdermal delivery has several advantages relative to oral delivery route as discussed in the next section.

1.7.3.2 Advantages of transdermal delivery

Drug delivery across the skin offers a non invasive, user-friendly alternative to conventional oral delivery because the skin presents a relatively large and readily accessible surface area for drug absorption (Benson, 2005). Transdermal drug delivery eliminates the variables such as pH, food intake and gastro-intestinal motility (Songkro, 2009). Secondly, transdermal delivery route circumvents the hepatic metabolism and is, therefore, suitable for drugs with a low bioavailability as well as eliminating the need for high initial drug dosages (Barry, 2001). Transport of drugs across the skin can also give a constant, controlled and sustained drug input, thereby decreasing the variations in drug plasma levels, thus reducing the side effects particularly of drugs with a narrow therapeutic window (Rivierea and Papich, 2001). Patient compliance may be increased due to the decreased frequency of dosing. This enhances the possibility of significantly improving the treatment of certain diseases as well as greatly increasing the patient's comfort due to possibility of self administration of the drug (Prausnitz and Langer, 2008). Transdermal delivery also circumvents HIV and other infectious disease transmission, which is possible when using hypodermic needles, as well as avoiding disposal challenges associated with these hypodermic injections (Prausnitz and Langer, 2008).

1.7.3.3 Limitations of transdermal delivery

Some drugs, however, may be inappropriate for transdermal administration such as those hindered by their physicochemical properties of being too large, highly polar or charged, having insufficient lipid solubility, or a tendency to cause direct skin irritation (Prausnitz and Langer, 2008). Such drugs may also have unfavourable pharmacokinetic or pharmacodynamic behaviour

such as having a very rapid clearance relative to achievable rate of skin delivery, first-pass cutaneous biotransformation, a requirement for intermittent high peak and low trough blood profiles or simply insufficient potency (Songkro, 2009). Therefore, candidates for passive transdermal delivery share three common properties, which include, effectiveness at relatively low doses, molecular mass less than 500 Dalton and lipophilicity. Due to the aforementioned drug penetration barrier of the intact SC, only potent drugs have been administered through the skin to date. Transdermal delivery has undergone three generations of development, which promises a greater possibility to deliver drugs of varied physicochemical properties in the near future (Prausnitz & Langer 2008). The use of penetration enhancers is a second generation of transdermal delivery method (Prausnitz & Langer, 2008). These penetration enhancers will be independently discussed in the next section.

1.8 Optimizing transdermal delivery

Diffusion of drugs across the skin is a passive process; hence compounds with low solubility and affinity for the hydrophilic and lipophilic components of the SC would, theoretically partition at a slow rate. Such difficulties may be overcome by addition of a chemical adjunct to the delivery system that would promote drug partitioning into the SC (Songkro, 2009). One long-standing approach for improving transdermal drug delivery uses penetration enhancers, also called sorption promoters or accelerants (Prausnitz and Langer, 2008). The role of penetration enhancer inclusion in transdermal formulations has been well documented (Songkro, 2009).

Optimization of drug permeation across the skin may be achieved using techniques that improve drug-vehicle interactions. By passing or removal of the SC and use of physical and electrically assisted methods are other techniques that may be used. Chemical penetration enhancers may be applied to modify the SC, thereby optimizing drug permeation.

Drug- vehicle based techniques include careful drug selection based on its physicochemical properties, use of pro drugs and ion pairs, modification of drug-vehicle interactions and chemical potential of the drug, use of eutectic systems, complexes, liposomes, vesicles and particles. On the other hand, techniques based on SC modification include, bypassing or removal of SC, physical and electrically assisted methods, as well as use of chemical penetration enhancers to cause SC lipid fluidization and hydration. However, the major focus of the discussion in the

current study will be on chemical penetration enhancers. Emphasis will be on penetration enhancers that were used in the current study, which include; a water based occlusive matrix patch system, dimethylsulphoxide (DMSO), eucalyptus oil, sodium oleate and vitamin E. Therefore, only a brief overview of the various categories of physical penetration enhancement techniques will be given in the next section followed by a discussion on the various classes of chemical penetration enhancers

1.8.1 Physical penetration enhancement techniques

The use of physical techniques to enhance drug penetration falls into various categories. The first category is based on drug and vehicle interactions (Barry 2001). Successful drug and vehicle interactions hinges on careful drug or pro-drug selection based on its physico chemical properties (Barry, 2001). Favourable physico chemical properties of a transdermally delivered drug or pro-drug include low relative molecular mass of less than 600 Dalton, adequate solubility in both water and oil and low melting point. The above mentioned properties may enhance the thermodynamic activity and partition coefficient of the drug in the SC thereby promoting drug permeation. Chemical potential of transdermally delivered drug must also be carefully considered as it has a bearing on the steady state drug flux in the SC (Songkro, 2009). Thermodynamic activity of a such drugs, may also be enhanced by addition of a co solvent, which evaporates at the skin surface, creating a supersaturated solution
(Moser et al., 2001). Formulations, such as these, require incorporation of a polymer to prevent crystallization of the supersaturated solution, so as to increase the metastability of the transdermal formulation. Using ion pairs, complex coacervates as well as eutectic systems as physical drug penetration enhancement techniques is also based on the manipulation of drug vehicle interactions. Use of ion pairs involves the addition of an oppositely charged species to a charged drug, leading to the formation of a neutral ion pair which can permeate and partition more readily through the SC. This complex dissociates in the viable epidermis, thereby rapidly releasing the parent charged molecule into the dermal and epidermal layers (Megwa et al., 2000a, Megwa et al., 2000b, Valenta et al., 2000, Sarveiya et al., 2004). Formation of eutectic mixtures is based on the principle that eutectic mixtures have a lower melting point, which serves to increase the solubility and skin penetration ability of the mixture (Stott et al., 1998).

Another category of physical drug penetration enhancement techniques is based on the use of vesicles and particles such as liposomes, transferosomes, ethosomes, and niosomes. Liposomes are phosphatidylcholine based colloidal particles that are formed as concentric biomolecular layers capable of encapsulating drugs (Touitou et al., 1994). Transferosomes, on the other hand are produced when a surfactant or an edge activator is incorporated onto a liposome. They are smaller than liposomes hence they have been shown to penetrate the SC at a faster rate than liposomes (Cevc, 1996, Cevc, 2003). Ethosomes are liposomes with high alcohol content. This makes their ability to penetrate deeper tissues and the systemic circulation to be enhanced (Dayan and Touitou, 2000, Touitou et al., 2000, Touitou et al., 2001). This is because ethanol fluidizes both the ethosomal lipids and those of SC, thereby allowing the drug to penetrate the disorganized lipid bilayers (Touitou et al., 2000, Touitou et al., 2001). Niosomes on the other hand are vesicles that are composed of nonionic surfactants. Niosomes are currently being investigated for the carrier of various drugs and cosmetics (Shahiwala and Misra, 2002). The above mentioned nanoparticles have recently been shown to enhance SC penetration due to increased skin hydration. The powderject system that fires high velocity solid particles through the SC into to lower skin layers using a supersonic shock wave of helium gas is an example of a physical drug permeation enhancement technique (Burkoth et al., 1999).

Bypassing or complete removal of the SC is another class of physical penetration enhancement techniques. The category is based on the concept of a microneedle array, ablation of SC and follicular delivery. A microneedle device is a jet propelled particle at high-velocity with compressed gas carrying drug particles into the skin (Cross and Roberts, 2004). This device is designed to penetrate the horny layer of the skin without stimulating nerves or breaking the horney layer (Langer, 2000, Barry, 2001). Ablation of SC involves removal of the horny layer using a laser, adhesive tape or chemical peels (Cevc, 1996). Drug uptake into the skin can, therefore, be measured using tape stripping protocol (Touitou et al., 1998). Follicular delivery refers to the use of pilosebaceous unit for drug delivery. The pilosebaceous unit comprises of the hair follicle, hair shaft and sebaceous gland and also referred to as the shunt drug delivery route.

Follicular delivery transports a drug directly to its target site, such as 'naked' DNA delivery for gene therapy (Hoffman, 2000).

The last category of physical penetration enhancement techniques includes physical and electrically assisted drug delivery methods. Ultrasound based techniques such as phonophoresis and sonosoresis, iontophoresis, electroporation, magnetophoresis and photochemical wave are examples of physical and electrically assisted physical techniques that aid drug permeation (Mitragotri, 2000). Ultrasound based techniques use ultrasound energy at low frequencies to massage topical formulation onto the SC as their penetration enhancement mechanism (Byl, 1995, Mitragotri, 2000, Mitragotri and Kost, 2000). Their mechanism of action involves cavitation. As the formulation is massaged onto the skin using ultrasound, cavities formed may collapse producing shock waves which may disturb the SC lipid packing, thereby increasing the volume available to aid drug penetration. They also cause an increase in lipid fluidity of the SC (Menon et al., 1994, Songkro, 2009). Iontophoresis on the other hand refers to the driving of charged molecules into the SC, via shunt routes due to application of a small direct current to a drug containing electrode in direct contact with the skin (Guy, 1998, Barry, 2001). Electroporation refers to the application of short micro- to milli-second electrical pulses of approximately 100-1000 V/cm to create transient aqueous pores in lipid bilayers (Prausnitz et al., 1996). The aqueous pores that are formed enable drugs to be transported through the horny layer (Prausnitz et al., 1996). Magnetophoresis and photochemical wave use the principle of delivering drugs in a pulsatile mode into the SC using magnetic or microchip based systems. This is due to that photomechanical waves or laser-generated stress waves cause transient permeabilisation of the SC. In this way, diamagnetic drugs or other molecules can be moved into the skin.(Langer, 2000). The major limitation of physical and electrically assisted physical drug permeation techniques is e that the safety margin for home use still needs to be evaluated (Barry, 2001). The safety concerns generally associated with physical penetration enhancement techniques has over the years promoted widespread use of chemical penetration enhancers.

1.9 Chemical penetration enhancers

Generally, chemical penetration enhancers have been widely investigated and evaluated, making them a safer and more practical alternative to the use of physical drug penetration enhancement techniques. Various chemical penetration enhancers will be discussed in the next section. Attention will, however, be given to the selected penetration enhancers that were incorporated into the transdermal formulation that was used in the current study. The selected chemical penetration enhancers have varied mechanisms of action as they belong to different classes of compounds. It is, therefore, imperative; to first discuss the mechanisms of action generally employed by various chemical penetration enhancers.

1.9.1 Mechanisms of action for chemical penetration enhancement

The release of a therapeutic agent from a transdermal formulation applied to the skin surface and its transport to the systemic circulation is a multistep process which involves dissolution within and release from the formulation, partitioning into the skin's outermost layer, the SC, diffusion through the SC, partitioning from the SC into the aqueous viable epidermis, diffusion through the viable epidermis and into the upper dermis, and finally the uptake into the local capillary network and eventually the systemic circulation. Mechanisms through which these enhancers increase drug penetration through the SC is best summarized in the lipid-protein partitioning (LPP) theory (Barry, 1991, Williams and Barry, 1991). The theory has proposed that penetration enhancement is via disruption of the intercellular lipid bilayer, interaction with intracellular proteins of the SC and the improvement of the partitioning of a drug, coenhancer, or cosolvent into SC.

Chemical penetration enhancers may act on the SC lipids because most enhancers have a polar head and a long alkyl chain (Trommer and Neubert, 2006). These chemical enhancers increase the permeability of the SC by simply fluidizing the lipid structure which results in a disordered structure, forming microcavities within the lipid bilayer, thereby increasing the diffusion coefficient of the drug or penetrant (Songkro, 2009). Other enhancers have the ability to penetrate the lipid bilayer and mix homogenously with the bilayer, while some others at high concentration pool within the lipid domains, creating permeable pores. These pores offer less

resistance for polar or hydrophilic penetrants (Ongpipattanakul et al., 1991). Another mechanism through which enhancers increase the penetrant's penetration is by causing perturbation of the enzyme systems responsible for the formation of the SC lipids (Barry 2001). An understanding of the structure activity relationships of chemical enhancers plays a major role in optimal penetration enhancement. Such enhancement is dependent on chain length, polarity, alkyl chain unsaturation and the presence of particular functional groups in the enhancer molecule (Aungst, 1989). Lipophilic excipients can, therefore, easily penetrate the lipid bilayer of the SC. The lipophilic excipients, however, also encounter opposition to penetrate the aqueous environment of the viable epidermis which can be managed by use of organic solvents like propylene glycol. The use of cholesterol and fatty acid inhibitors can also increase excipient or permeant penetration. The clinical applications of most penetration enhancers, however, is limited by that they also cause irritation to the skin (Kanikkannan and Singh, 2002). In summary, the penetration enhancers, penetrate the lipid bilayer, rotates, vibrates, translocates thereby forming microcavities and increasing volume available for drug diffusion.

Protein modification is another mechanism of action for some chemical enhancers. The intracellular matrix of SC consists of corneocytes, which are made up of keratin filaments. A chemical enhancer penetrating the intracellular matrix may interact and bind to these keratin filaments thereby disrupting the order existing inside the corneocyte (Benson, 2005). This has an effect of increasing the diffusion coefficient and permeability of the penetrant. Modification of the peptide/protein molecules in the lipid bilayer domain is the other mechanism suggested. However, all these enhancers, as already mentioned have a limitation of inducing skin irritancy.

Finally, chemical enhancers may promote drug partitioning in the SC, as their mechanism of action. Promotion of partitioning has an overall effect of enhancing the ability of the penetrant to partition into and solubilise within the SC. The chemicals do this by shifting the solubility parameter of the skin, moving it closer to that of the penetrant, thereby increasing its solubility and flux (Liron and Cohen, 1984a, Liron and Cohen, 1984b). Additional chemical penetration enhancer mechanisms may generally include action of the enhancer on desmosomal connections between corneocytes as well as altering metabolic activity within the skin to aid drug penetration across the SC (Benson, 2005). In summary, there are many potential sites and modes of action

that have been identified for skin penetration enhancers such the intercellular lipid matrix in which the enhancers may disrupt the packing motif, the intracellular keratin domains or through increasing drug partitioning into the tissue, in which case the enhancer, may act as a solvent for the drug within the membrane.

Generally, the ideal or desirable properties of penetration enhancers include that that they should be non-toxic, non-irritating and non allergenic (Trommer and Neubert, 2006). They should ideally work rapidly, and the activity and duration of effect should be both predictable and reproducible. They should have no pharmacological activity within the body such as any ability to bind to receptor sites (Prausnitz and Langer, 2008, Songkro, 2009). The penetration enhancers must work unidirectionally by allowing therapeutic agents into the body whilst preventing the loss of endogenous material from the body (Barry, 1991). When removed from the skin, barrier properties should return both rapidly and fully. The penetration enhancers should be appropriate for formulation into diverse transdermal preparations, thus should be compatible with many different drugs (Rivierea and Papich, 2001). They should be cosmetically acceptable with an appropriate skin 'feel'(Barry, 1991). However, none of the available chemical penetration enhancers possess all the properties, hence need for continued study in this area is still of prime importance.

There are various classes of chemical penetration enhancers, such as phospholipids, pyrrolidones, surfactants, amides, polyols, esters, alcohols, oxazolidines and epidermal enzymes. Polymers, lipid synthesis inhibitors, azone and its derivatives as well as biodegradable enhancers are additional classes of chemical penetration enhancers. The discussion in the next section, however, will be limited to the classes of the chemical penetration enhancers that were used in the current study. The classes of chemical penetration enhancers that were incorporated into the transdermal formulation used in current study include water, oleic acid which is a fatty acid, eucalyptus oil which is an essential oil, vitamin E which is an antioxidant as well as DMSO, which belongs to the class of sulphoxides.

1.9.1. 1. Water

Water has been shown to be the safest and widely used penetration enhancer for both hydrophilic and lipophilic drugs and pemeants (Barry, 2001). The penetration enhancement mechanism of water is via increasing the hydration and permeability of the horny layer(Scheuplein and Blank, 1973). Water content of the SC varies depending on the humidity of the external environment. Water in the SC exists in different states, such as 'bound' water which is associated with structural elements of this layer. The remaining is 'free' water, which is available to act as a solvent within the membrane for polar or hydrophilic drugs (Kalia et al., 2000). Additionally, the skin contains the natural moisturizing factor (NMF). This is a humectants mixture of the hygroscopic amino acids and their derivatives, including salts that are naturally found in the SC. The purpose of this NMF is to retain skin water in conjunction with functional groups of keratin proteins that bind water molecules. The overall effect is maintenance of skin pliability. Increased water content of the skin increases SC hydration which in turn causes swelling and therefore opening the SC membrane lipid structure. Clearly, free water molecules therefore, alter the solubility of the drug, thereby modifying drug partitioning from its vehicle, into SC. Logically, this is expected for hydrophilic drugs, and not lipophilic pemeants (Barry, 2001, Songkro, 2009). Water may also use other mechanisms other than just distorting the lipid bilayer to aid drug penetration. The possible mechanisms varies from the corneocytes taking up water and swelling to creation of a continuous aqueous pore pathway comprising lacunar dormains that are embedded in the lipid bilayers. This pathway enhances drug permeation through the SC (Songkro, 2009). Caution must, however, always be taken whenever rodents hairless skin are used to mimic the human skin for transdermal delivery of various drugs. This is because, upon raising hydration of the SC, the rodent skin increases drug flux by higher magnitude than the human skin (Bond and Barry, 1986, Bond and Barry, 1988).

For transdermal drug delivery systems, hydration is increased by occlusion of the skin using plastic films; paraffin, oils, water-in-oil or oil-in-water emulsions as well as waxes that are used as components of ointments (Songkro, 2009). A water-in-oil system prevents transepidermal water loss while an oil-in-water emulsion donates a water molecule to the SC. Various occlusive films have been designed and experimentally tested. Of these, are films that used plastic or oily

vehicle that were shown to achieve optimal SC hydration thereby giving maximal pemeant penetration (Barry, 2001).

1.9.1.2. Fatty acids

Oleic acid belongs to this class of penetration enhancers. Long chain fatty acids have reportedly been used as penetration enhancers (Larrucea et al., 2001). Extensive studies by Aungst, concluded that saturated alkyl chain lengths of C10–C12 attached to a polar head group yield potent enhancers, while C18 is the optimum chain length for unsaturated alkyl chains (Aungst, 1989, Funke et al., 2002). The *cis* configuration of unsaturated alkyl chains disturbs the lipid packing motif more than the *trans* conformation. Various drugs have so far been delivered with the aid of fatty acids as a penetration enhancer (Funke et al., 2002). Oleic acid has also been shown to enhance penetration of various drugs at minute concentrations, in addition to exhibiting synergistic effects when used in conjunction with PG (Sintov et al., 1999). Analogues of fatty acids also possess penetration enhancement activity (Takahashi et al., 2002). Fatty acids interact and modify the lipid domains as their mode of action as well as existing in this layer as pools or a separate phase, thereby facilitating permeation of hydrophilic drugs. When used at high concentrations, oleic acid tends to phase separate, meaning that they tend to pool within the lipid domains forming permeable pores. These pores enhance transport of various molecules (Barry, 2001). Studies have evaluated the effects of physico chemical properties of many fatty acids, such as their chain length, polarity, unsaturation of the alkyl chain as well as presence of special functional groups in aiding drug penetration (Kanikkannan et al., 2000).

1.9.1.3. Essential oils, terpenes and terpenoids

Eucalyptus oil belongs to this class of penetration enhancers. The mechanism of action of this class of compounds, involves the disruption of the elegant lipid bilayer structure as well as forming separate pools within the bilayer to create hydrophilic channels (El Maghraby, 2008). Terpenes, which are found in essential oils, have also been shown to act as penetration enhancers in addition to their general use as flavourants and fragrance agents (Williams and Barry, 1991,

El-Kattan et al., 2000, Cal et al., 2001, Jain et al., 2002, Monti et al., 2002, Morimoto et al., 2002).

1.9.1.4 Anti-oxidants

Vitamin E belongs to this class of penetration enhancers. Generally, it is believed that Vitamin E decreases the gel state order of phospholipid membranes (Thiele and Ekanayake-Mudiyanselage, 2007). Vitamin E intercalates within the lipid bilayer, to disorder gel phase lipids without causing any irritation to the skin (Triverdi 1995). The overall effect is to increase the permeability of the skin, aiding drug penetration into SC (Thiele and Ekanayake-Mudiyanselage, 2007). In addition to drug penetration enhancement and antioxidant properties, vitamin E also possesses emollient properties (Triverdi 1995)

1.9.1.5 Sulphoxides

DMSO belongs to this class of chemical penetration enhancers. DMSO is generally known to be a powerful aprotic solvent with a unique ability to hydrogen bond with itself rather than with water (Songkro, 2009). It is a colourless, odourless and hygroscopic compound. As such, in most pharmaceutical applications, DMSO is generally used as a ''universal solvent'', or as a co-solvent in formulations or alone (Barry, 2001). DMSO can act as a penetration enhancer for both hydrophilic and lipophilic permeants. The use of DMSO, as a penetration enhancer is concentration dependant, with concentrations above 60% recommended whenever it is used as a co-solvent in drug formulations (Songkro, 2009). DMSO has also been shown to have a rapid mode of action (Barry, 2001). The compound has inherently multiple, but complex mechanisms of action. DMSO reportedly denatures proteins, changing their structure from a helical conformation to an h sheet (Barry, 2001). Based on its physicochemical structure, the DMSO molecule interacts with the head groups of the lipid bilayer thereby distorting its packing geometry. Such an interaction is possible because DMSO is highly polar (Benson, 2005). Furthermore, in the SC membranes, DMSO, may promote drug partitioning from its vehicle into this universal solvent. DMSO also penetrates the lipid bilayer, rotates, vibrates, translocates

thereby forming microcavities and increasing volume available for drug diffusion (Prausnitz and Langer, 2008).

There are numerous compounds such as dimethylacetamide and dimethylformamide whose structure is related to DMSO. However, as is the case with DMSO, such analogues may also cause irreversible SC damage (Southwell and Barry, 1983). Unlike DMSO, decylmethylsulphoxide, does not cause irreversible SC damage although its effects are also concentration dependent, just like its parent molecule (Barry, 2001). Various investigators have reported that DMSO continues to be used to deliver various drugs to date (Barry et al., 1984). The major limitation to the use of DMSO is that at concentrations above 60%, it may cause erythema as well as denaturation of SC proteins(Pathan and Setty, 2009). Furthermore, the metabolite, dimethylsulphide produces a foul odour on the breath (Songkro, 2009). DMSO possibly has greater effects on the rodent skin than the human skin. This is because the hairless, rodent plasma membrane is more fragile, yet DMSO is a relatively harsh compound. It is common in the pharmaceutical industry to produce transdermal formulations that have more than one penetration enhancer, in order to benefit from the synergistic effects of their combinations as discussed in the next section.

1.9.1.5 Synergistic effects of penetration enhancers

Transdermal formulations generally incorporate two or more penetration enhancers from different classes of chemical compounds in order to benefit from synergistic effects of such combinations (Songkro, 2009). Reports indicate that various combinations of enhancers have multiplicative or synergistic effects (Harrison et al., 1996, Barry, 2001). In some formulations, synergistic effects of combining physical and chemical penetration enhancers has also been employed (Barry 2001). Various enhancement strategies have varied effects on the transdermal drug delivery (TDD) formulation or the skin in general. It is vital to note at this stage, that caution must always be exercised in the choice of a penetration enhancer for any drug delivery system. This, is due to the fact that they may cause skin irritancy and in some rare cases, toxicity (Sloan et al., 1986). This has limited the clinical application of most enhancers; hence safer enhancers must still be sought.

In summary, synergistic effects of various penetration enhancers may alter the release of a drug from a formulation, depending on the chemical potential of the drug as well as causing SC lipid fluidization. Some enhancers act as biochemical modulators in the viable epidermis, while vasoactive drugs modulate blood flow rate, thereby influencing rate of drug absorption into systemic circulation. The transdermal formulation used in this study employed the synergistic effects of combining chemicals in different classes of penetration enhancers, thereby benefiting from their varied modes of action to aid the penetration of CHQ across the SC (Songkro, 2009).

1.9.1.6 Transdermal drug delivery systems

Transdermal delivery systems (TDDs) are drug containing technical devices that form a temporal unit with the skin (Barry 2001)**.** The majority of TDDs act through increasing SC hydration to open up the pores of the horny layer, thereby aiding drug penetration (Roy et al., 1996, Venkatraman and Gale, 1998, Wokovich et al., 2006, Padula et al., 2007, Mishra et al., 2009). There are various TDDs that increase the hydration status of the SC, namely, lipophilic materials, emulsifying bases, water oil emulsions, oil-water emulsions, absorption bases, moisturizing factors, humectants and powders, occlusive dressings, and occlusive patches (Barry 2001). Lipophilic based TDDs consist of paraffin, oils, fats, waxes, fatty acids, alcohols, esters and silicones. Combining these constituents in lipophilic based TDDs prevents water loss from the SC, thereby producing full hydration. The overall effect is therefore an increase in skin permeability. The other TDDs attain full SC hydration through various mechanisms ranging from donating water molecules to preventing water loss as seen in occlusive patches (Songkro, 2009). TDDs based on the concept of occlusion include occlusive dressings and occlusive patches. These TDDs have been shown to effectively prevent water loss from the SC, thereby having an effect of causing full hydration of the skin. An occlusive dressing is made up of a plastic film, and an imperforated waterproof plaster where as the occlusive patch is an example of transdermal patches(Prausnitz and Langer, 2008). The current study used an occlusion based TDDs as will be discussed in the next section.

1.9.1.6.1 Occlusive transdermal drug delivery patch systems

Generally, a TDDs consists of several components, including the active ingredient, backing membrane, pressure-sensitive adhesive and the permeation enhancer (Barry, 2001). Examples of occlusive TDDs include the reservoir systems and the matrix systems (Rhee et al., 2008). Transdermal patches come in different forms and designs such as single layer drug in adhesive form, multilayer drug in adhesive form, vapour patches, reservoir patches and transdermal patches. Reservoir patch systems consist of drug deposits surrounded by insoluble polymer membranes, which are the control release elements (Roy et al., 1996, Venkatraman and Gale, 1998, Wokovich et al., 2006, Padula et al., 2007, Mishra et al., 2009). The mechanism controlling release of the drug molecule is diffusion. Examples of polymers used in this system are ethylcellulose, hydropropyl cellulose, waxes, silicone derivatives and ethyl vinyl acetate .On the other hand, matrix patch systems are those in which the drug is intimately mixed with an inert polymer. Drug release in these systems also occurs by diffusion, but is controlled by the properties of the polymer. These are also referred to as monolithic systems (Rhee et al., 2008). Matrix systems can either be hydrophobic or hydrophilic. The occlusive matrix system has several advantages which include that the entire system can be made to be thin and elegant. The matrix system can also be made very comfortable to wear. Additionally, matrix systems exhibit flexibility in the choice of backing layers (Venkatraman and Gale, 1998). Matrix TDDs can be made up of synthetic or natural polymers such as pectin, which was the polymer of choice for this study. The chemistry of pectin, as well as how its physicochemical properties are modified by other components of the matrix patch will be discussed in the next section. Such modifications include varying degrees of pectin methylation, amidation and addition of Ca^{2+} ions.

1.10 Transdermal delivery polymers

1.10.1 Chemistry of pectin

Pectin (polygalacturonic acid) is an anionic polysaccharide present in the cell wall of most plants (Ridley et al., 2001). It is non-toxic, almost totally degraded by colonic bacteria and is not digested by gastric or intestinal enzymes (Cummings et al., 1979). Pectin structure is

heterogeneous with respect to its chemical structure and molecular weight (Ridley et al., 2001). The poly (d-galacturonic acid) moieties in pectin are bonded via a 1-4-glycosidic linkage (Ridley et al., 2001, Matia-Merino et al., 2004, Lofgren et al., 2006). Pectin also contains neutral sugars such as L-rhamnose, which are either inserted in or attached to the main chains. The carboxyl groups in pectin molecules are partially in the methyl ester form. Some of the carboxyl groups may be converted to carboxamide groups, when ammonia is used in the process of de-esterification, producing amidated pectin (Ridley et al., 2001). The properties of pectin are dependent on the degree of amidation (DA) and degree of methylation (DM), which denotes the amide and methyl groups attached to carboxyl and hydroxyl groups of the pectin molecule respectively. Pectin forms water-insoluble complexes with several drugs and may be a useful additive for sustained-drug release preparations (Ridley et al., 2001). Colonic bacteria are able to degrade pectin, hence there has been renewed interest to investigate use of pectin as a carrier for drugs whose target delivery site is the colon (Rubinstein et al., 1993, Munjeri et al., 1998, Musabayane et al., 2000b). The possible use of pectin in transdermal delivery formulations has also been suggested (Musabayane et al., 2003). The gelling properties of pectin are influenced by various factors, which include the DA, DM, pH of the solution, temperature as well as the presence of cations such as calcium ions (Ridley et al., 2001). The various ways that can be used to optimize the gelling properties of pectin are discussed in the next section.

1.10.2 Optimisation of gelling properties of pectin

Gelling of pectin can be induced by acid or by cross-linking with calcium ions (Sriamornsak, 1998). Ionisation of carboxylate groups of pectin is repressed in an acidic environment or when the pH of the solution is low. Unionized pectin carboxylate groups do not repel each other over their entire chains, and thus can associate over a portion of their chains to form acid pectin gels (Matia-Merino et al., 2004). Acidic solutions reduce the hydration of pectin, meaning there are fewer water molecules that are incorporated into the pectin interchain entanglements. The subsequent increases in the pH of the solution causes ionization of the polycarboxylate groups, enabling them to react with cations such as calcium ions to form calcium pectinate (Sriamornsak, 1998). The interaction of calcium ions and the carboxylate groups in pectin involves intermolecular chelate binding of the cation leading to the formation of macromolecular

aggregates (Rubinstein et al., 1993). In the colon, calcium pectinate can readily be degraded by bacterial pectinases which are activated by the presence of calcium ions in the pectin molecule. Generally, the amount of calcium ions added into the pectin gels also has an impact on the strength of the gels and the release of incorporated drugs

1.11 Basis of the present study

We speculated that use of pectin CHQ matrix patches may eliminate the development of transiently high initial systemic drug concentrations associated with oral CHQ administration. Such a sustained, controlled, slow release of the CHQ from the matrix patch may circumvent development of the adverse effects of CHQ on glucose homeostasis, renal and cardiovascular function that are associated with oral delivery. We also envisaged that the sustained, slowly released CHQ concentration from pectin CHQ matrix patch eliminates *P. falciparum* parasites. There are currently no reports in literature that have shown the effects of transdermal application of pectin CHQ matrix patches on glucose homeostasis, renal and cardiovascular function in a malaria rat model. Therefore, the present study, was designed to compare the effects of oral and transdermal delivery of CHQ on glucose homeostasis, renal and cardiovascular function in a malaria rat model were investigated.

1.12. Aims of the study

The main objective of the current study was to compare the effects of transdermal CHQ patch and oral chloroquine in the management of malaria as assessed by the ability to clear parasites of *P. berghei* infected rats. The other aims were to investigate and distinguish between the patho physiological effects of malaria and CHQ treatments on blood glucose and plasma insulin concentration, renal and cardiovascular function in male Sprague-Dawley rats.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1   Drugs and chemicals

Drugs and chemicals were sourced as indicated:

Grindsted pectin LA 410 (Danisco, Palackého, Czech Republic); chloroquine diphosphate ($C_{18}H_{26}CIN_3 \cdot 2H_3PO_4$), sigmacote, dimethyl sulphoxide (C_2H_6SO), anthrone and glycogen powder, type II, from oyster, giemsa stain (Sigma-Aldrich Chemical Company, Missouri , St Louis, USA); calcium chloride ($CaCl_2$), potassium hydroxide (KOH), sodium sulphate (Na_2SO_4), sodium hydroxide (NaOH), dimethyl sulphoxide (DMSO), d-glucose, potassium dihydrogen phosphate (KH_2PO_4) and 95% ethanol (C_2H_5OH) (Merck Chemicals (PTY) LTD, Johannesburg, South Africa); vitamin E (Pharma Natura (PTY) LTD, Johannesburg, South Africa); eucalyptus oil (Barrs Pharmaceutical Industries cc, Cape Town, South Africa); diethyl ether ($C_4H_{10}O$) (NT Laboratory Supplies (PTY) LTD, Johannesburg, South Africa); sulphuric acid (H_2SO_4) (BDH Chemicals LTD, Poole, England), halothane (Fluorothane®, AstraZeneca Pharmaceuticals (PTY) LTD, Johannesburg, South Africa), and The Ultrasensitive Rat Insulin ELISA kit -(DRG Instruments, GmbH, Marburg, Germany). All chemical reagents were of analytical grade.

2.2. Ethical consideration

Ethical clearance was obtained from the University of KwaZulu-Natal's Ethics committee (reference 018/09 Animal; 017/10 Animal and 10/11/Animal see Appendices I, II and III).

2.3. Animals

Male Sprague-Dawley rats (90-150 g) bred and housed in the Biomedical Resource Unit of the University of KwaZulu-Natal, Westville campus were used in the study. The animals were maintained under standard laboratory conditions of constant temperature (22±2 °C), CO_2 content

of <5000 p.p.m., relative humidity of 55±5%, and illumination (12 h light/dark cycles) and the noise levels of <65 decibels. The animals had free access to standard rat chow (Meadows, Pietermaritzburg, South Africa) and water.

2.4 Methods

2.4.1 Preparation of the pectin-CHQ matrix patch

The pectin-CHQ matrix patch was prepared with slight modifications according to the method previously described by (Musabayane et al., 2003). Briefly, pectin (4g) was dissolved in 100ml de-ionised water with agitation at 700 rpm using a mixer (Scientific Engineering cc, Johannesburg, 51A Richard rd, South Africa). Various doses of chloroquine disphosphate, 5g, 10g and 20g were then added with further mixing and agitation in separate beakers that were placed in a water bath at 37^OC for 30 minutes. Subsequently, dimethyl sulphoxide (3ml, vitamin E (1.5ml) and eucalyptus oil (1.5ml) were added. Following 4 hours of further mixing and agitation, an aliquot of (10ml) was transferred to a petri dish and frozen at -5^oC for 18 hours. After freezing, a 2% $CaCl_2$ was added on top of the frozen pectin and left to stand at room temperature for 10 min to allow for cross linking. The patches were then stored in a refrigerator at 2^oC until use. Before the commencement of the experiments, the dorsal region of the rat was shaved for the application of the pectin CHQ matrix patch. The effects of transdermal CHQ were assessed by placing patches on the shaved area and securing them in place with adhesive hydrofilm (Dischem, Cnr of Le Roux & Stay rd Glen Austin, South Africa) and rat jackets (Braintree, Scientific, inc , Massachusetts, USA). The control group was sham applied with CHQ free pectin matrix patch.

2.4.2. Determination of % CHQ incorporation

Pectin CHQ matrix patch of known volume and surface area was dissolved in 100ml of Sorensen buffer (pH 7.4), with shaking for 30 minutes at 700rpm in order to speed up the dissolution process. 2 ml of the mixture was withdrawn to measure chloroquine spectrophotometrically

(Shimadzu Corporation, Kyoto, Japan) at 343nm. The blank contained CHQ free pectin hydrogel patches dissolved in Sorensen buffer (pH 7.4) (Musabayane et al., 2003).

2.5. Induction of malaria

Malaria was induced in male Sprague-Dawley rats (90-150g) by a single intra-peritoneal injection of *P berghei* (10^5 parasitised RBC). (Gumede et al., 2003). The rodent CHQ susceptible *P. berghei* parasite was supplied by Professor Peter Smith (University of Cape Town, Division of Clinical Pharmacology, South Africa). Control animals were injected with the phosphate buffered saline vehicle. Successful malaria induction was confirmed by microscopic examination of Giemsa stained thin smears of the rat tail blood. Percentage parasitaemia of greater than or equal to 20% was considered as a stable malaria state before commencing any experimental procedures.

2.6. Experimental design

Acute effects of CHQ on blood glucose (0-4h) and short-term effects on renal and cardiovascular functions (3 weeks) were monitored in separate groups of non-infected and *P. berghei* infected male Sprague-Dawley rats (n= 6 in each group).

2.7. Acute studies

2.7.1 Oral glucose tolerance test (OGTT)(protocol

OGT responses were carried out as previously described (Musabayane et al., 2007) with slight modifications. The animals were divided into non infected and *P. berghei* infected groups. The non infected and *P. berghei* infected groups were further divided into, control group, oral CHQ and patch treated groups. The treatment protocol for all groups was as follows; the animals were fasted for 18 h, followed by measurement of blood glucose (time 0) and subsequent loading of glucose (0.86 g/kg, p.o.). OGT responses were monitored in separate groups of animals treated with various doses of oral CHQ (30, 60 and 120 mg/ kg or pectin-CHQ matrix patch (29, 53 and 72 mg/kg). CHQ diphosphate solution was prepared by dissolving CHQ diphosphate powder in deionised water and given to the rats by means of a soft tube passed orally into the stomach

(Musabayane et al., 1994). Oral CHQ control animals were given de-ionised water, while CHQ matrix patch control animals were sham applied with CHQ free pectin matrix patch. Blood glucose concentrations were measured over a 4-hour period; every 15 minutes for the first one hour and subsequently every hour. Blood was obtained via tail prick for blood glucose concentration measurements (Acsensia Elite Blood Glucose Testing Strips, Bayer®) and Bayer's Glucometer Elite® (Elite (Pty) Ltd, Health Care Division, Isando, South Africa).

2.7.2. Effects of CHQ on plasma insulin

Plasma CHQ and insulin concentrations were determined in blood samples of separate groups of non- infected animals treated with oral CHQ (60mg.kg b.wt) and pectin matrix patch (53mg/kg). The orally treated group was given CHQ diphosphate solution twice daily, 8 hours apart for 5 consecutive days. Treatment with pectin CHQ matrix patch was a once off application of the patch on the first day of the 5 day treatment period at 09h00. The patch was placed on the shaved area of the rat back neck and was left on for 5 day treatment period. The animals (n=6) were sacrificed at the end of 0.25; 0.5; 1; 3 and 5days via halothane inhalation (Fluorothane®, Astra Zeneca pharmaceuticals (Pty) LTD). Blood was collected by cardiac puncture into pre-cooled heparinized tubes for blood glucose concentration and plasma insulin determination. The collected blood was centrifuged (Eppendorf centrifuge 5403, Schwerte, Germany) at 4 °C, 3000g for 15 minutes and separated plasma was stored at -70 °C in a Bio Ultra freezer (Snijers Scientific, Tilburg, Netherlands) until insulin assay. Effects of CHQ on plasma insulin on *P. berghei* infected animals was not done in the current study.

2.8 Short term studies

Male Sprague-Dawley rats (90g-120g) were used in the non-infected and *P berghei* infected, control and treated groups. The animals were housed individually in Makrolon polycarbonate metabolic cages (Techniplasts, Labotec, South Africa) for three weeks at the Biomedical Resource Unit, University of KwaZulu- Natal, (n=6 in each group) with free access to water and standard rat chow *ad libitum* (Meadows, Pietermaritzburg, South Africa). The rats were maintained on a 12 h dark/light cycle.

The effects of oral CHQ treatment (60mg/kg) and pectin CHQ matrix patch (53 mg/kg) were investigated in both the non infected and *P berghei* infected animals. The 21 day studies were divided into pre-treatment (days 0-7), treatment (days 8-12) and post treatment (days 13-21) periods. The separate groups of animals were treated with oral CHQ and pectin CHQ matrix patches as described in section 2.7.2.

2.8.1 Short-term effects of CHQ treatments on:

2.8.1 1. Parasitaemia

Daily malaria parasite density was measured in separate groups of *P berghei* infected animals throughout the 21 day experimental period. Thin blood films stained by Giemsa method as previously described by Gumede et al., (2003) were examined. Briefly, a small incision in the tip of the rat tail was made followed by placing a small drop of blood on a microscope slide. This was smoothly and uniformly smeared using a second microscope slide. The blood film on the slide was subsequently dried in a stream of warm air, fixed in methanol for 30 s and then stained in Giemsa stain for 10–45 min. Finally the blood film was dried in air and observed using an Olympus microscope (Olympus cooperation, Tokyo, Japan) with a x50–x100 oil immersion objective. Extreme caution was taken to distinguish parasites from inclusions in RBC such as Howell-Jolly bodies, platelets on top of RBC, or from precipitated stain. An eyepiece graticule was used to obtain accurate counts. Counting of 5 microscope fields was followed by calculation of the percentage of infected RBCs.

2.8.1 .2 Physico metabolic changes

The animal weights, and the amounts of food and water consumed were measured at 09h00 every 3rd day during the pre treatment and post treatment periods, and daily during the treatment period. Blood glucose concentration was measured 6 hours after treatment in all groups of animals using blood glucose testing strips (Acsensia Elite Blood Glucose Testing Strips, Bayer®) and Bayer's Glucometer Elite® (Elite (Pty) Ltd, Health Care Division, Isando, South Africa). The 24 h urine volume and urinary urea, creatinine and electrolytes (Na$^+$, K$^+$) outputs

were also measured. Haematocrit was measured using heparinised capillary tubes. Briefly, blood was collected via a tail prick. The sealed capillary tubes were then centrifuged for 3 minutes at 3000g with a Micro-haematocrit centrifuge (346 MSE Centrifuge Manufacturer, London, UK). Haematocrit was then determined by calculating the percentage of the total blood volume to packed cell volume. The measurements were done at 09h00 every 3rd day during the pre treatment and post treatment periods, and daily during the treatment period.

2.8.1.3 Mean Arterial Pressure (MAP)

MAP was monitored every 3rd day at 09h00 in separate groups of conscious Sprague Dawley rats for three weeks using the non invasive tail cuff method (IITC Model 31 Computerized Blood Pressure Monitor, Life Sciences, Woodland Hills, California, USA) as previously described (Musabayane et al., 2007). During the treatment period, MAP was measured daily at 09h00. The unit comprises the IITC hardware system that utilizes an automatic scanner pump, photoelectric tail cuff sensors and an amplifier. The blood pressure of the tail vein of the animal is measured and the output is recorded in form of a chart that is displayed on the connected computer monitor. To ensure accuracy and consistency, the equipment was calibrated each day before commencing MAP measurements. The animals were warmed in an enclosed chamber (IITC Model 303sc Animal Test Chamber IITC, Life Sciences, Woodland Hills, California) for 30 minutes at ±30°C before taking three blood pressure recordings of each animal as previously described by Gondwe et al., (2008).

2.8.2 Terminal studies

Separate groups of non fasted conscious animals (n=6) were sacrificed on days 0, 7, 8, 9, 10, 12, 14 and 21, at 24 hours after the last treatment for oral CHQ administration and after a once off patch application on the first day of treatment. The sacrifice procedure was performed by halothane inhalation in an anaesthetic chamber with 100 mg/kg of halothane (Fluorothane®, Astra Zeneca 2002) pharmaceuticals (Pty) LTD) for 3 minutes. Blood was collected by cardiac puncture into pre cooled heparinised tubes and centrifuged for 15 minutes at 3500g and 4 °C to separate the plasma (Eppendorf centrifuge 5403, Germany). The plasma samples were stored in a

Bio Ultra freezer (Snijers Scientific, Tilburg, Netherlands) at -70 °C until assayed. Concentrations of plasma electrolytes (Na^+, K^+, Cl^-), urea, creatinine, CHQ and insulin were measured. Plasma concentrations of the lipid profile parameters were also measured.

The harvested liver, gastrocnemius muscle tissue (1-1.5g), kidney, heart and spleen were weighed gravimetrically (Mettler balance PC 180-insruments, Protea Laboratory Services, South Africa) and snap frozen in liquid nitrogen. The liver and gastrocnemius muscle tissue (1-1.5g) used for glycogen determination were immediately placed on ice (Ngubane et al., 2011).

2.9. Laboratory analyses

2.9.1. Biochemical measurements

2.9.1.1 Determination of plasma CHQ concentration

Following the treatment of separate groups of animals with oral CHQ and pectin CHQ matrix patch, measurement of plasma CHQ concentration was conducted. Sigmacote was used to coat all glassware to prevent adsorption of CHQ to the glass surfaces. Plasma CHQ determination was carried out by means of a double extraction process as previously described by Musabayane et al., 2000 (Musabayane et al., 2000) with slight modifications. Briefly, 0.2ml of plasma sample was added to a 250ml separator flask followed by 1ml of 25% KOH and 10mls of diethyl ether. The solution was then mixed and allowed to stand for 2 minutes to allow the immiscible layers to separate followed by acidification of the solution using. 2ml of 1% H_2SO_4. Absorbance of resultant aqueous solution was then read using a spectrophotometer (Ultraviolet Novaspec II spectrophotometer (Biochrom LTD, Cambridge, England) against a plasma blank at 343nm.

2.9.1.2 Electrolytes measurements

Determination of urine and plasma Na^+, K^+, CI, urea and creatinine concentrations were measured using the Beckman Coulter (Synchron LX20 Clinical Systems, Fullerton, Carlifornia, USA). The principle of creatinine estimation is based on reaction of creatinine and sodium picrate to form creatinine picrate while that of urea estimation is based on the hydrolytic

degradation of urea in the presence of urease. For all the urea and electrolytes measurements, standard kits and reagents were supplied by Beckman Coulter, Dublin, Ireland. Glomerular filtration rate (GFR) was assessed by creatinine clearance (C_{cr}). This parameter was calculated using a standard formula based on the measurements of the plasma and urinary concentrations of creatinine and urine flow rate on different days on which separate groups of animals were sacrificed. The samples were prepared by diluting and mixing using a vortex machine (VELP Scientifica, Usmate (MB), Italy). To measure both urine and plasma creatinine no dilution was necessary.

2.9.1.3 Insulin assay

Standard enzymatic methods were used to determine plasma insulin concentration. The assays were performed on an ultra sensitive rat insulin ELISA kit (DRG diagnostics EIA-2943 GmbH, Marburg, Germany), using reagents supplied by the manufacturer

Principle of the insulin assay

The kit components included a 96 well plate coated with mouse monoclonal anti-insulin, standards, enzyme conjugate, enzyme conjugate buffer, wash buffer, substrate 3,3',5,5'-tetramethylbenzidine (TMB) and a stop solution. This kit is a solid phase two-site enzyme immunoassay which is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation, insulin in the sample reacted with peroxidase-conjugated anti-insulin antibodies and anti-insulin anti-bodies bound to a microtitration well. Unbound enzyme labeled antibody was removed by a sample washing step. Detection of the bound conjugate was done by a reaction with 3,3'-5,5' tetramethylbenzidine. The reaction was stopped by adding sulphuric acid (0.5M) to give a colorimetric endpoint that is read in a spectrophotometer at 450nm. Each determination was performed in duplicate for all standards and samples. The lower and upper limits of detection were 139 pmol/1 and 960 pmol/1, respectively. The intra-assay analytical coefficient of variation ranged from 4.4 to 5.5% and the inter-assay coefficient variation from 4.7 to 8.9%.

2.9.1.3 Glycogen determination

Hepatic and muscle glycogen concentrations were determined as previously described by (Ngubane et al., 2011) . Briefly, the tissue samples of 1-1.5g were homogenized in 2ml of 30% KOH (30g/100ml) and boiled at 100°C for 30min. Subsequently, $\pm$ 0.15ml of 10% Na_2SO_4 (10g/100ml) was added to the tissue homogenates, which were then vortexed. 2μl of each sample was added to 2μl of 95% of ethanol, 1ml of de-ionised water and 4ml anthrone reagent (0.5g in 250ml of concentrated H_2SO_4) to extract the glycogen from the liver and muscle sample. Blanks were prepared in a similar process as tissue samples except that the sample was replaced with 2μl of de-ionised water. Glycogen powder from oyster, type II was used to prepare standards (10-2000 mg/1) which were also treated in the same way as tissue samples described above. All solutions were then vortexed and boiled for 10min before measuring absorbance at 540nm using a UV mini-1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). A glycogen standard curve that was prepared as previously described by (Ngubane et al., 2011), was used for the extrapolation of all tissue glycogen concentrations.

2.9.1.4 Lipid profile analysis

Standard enzymatic methods were used to determine the plasma lipid profile parameters. The assays were performed on the Labmax Plano Chemistry analyzer, (Labtest Av. Paulo Ferreira da Costa, 600, Brasilia, Brazil) using reagents supplied by the manufacturer

Plasma enzymatic determination of total cholesterol and triglycerides were followed by measurement of HDL-cholesterol using an HDL-c precipitating reagent set (Warnick and Wood, 1995). LDL-cholesterol and VLDL-cholesterol concentration were then calculated using the Friedewald formula:

- LDL-c = [Total cholesterol (HDL-Triglyceride)/2 mmol] (Friedewald, Levy and Fredrickon, 1972).

- VLDL-c = Total cholesterol – (LDL-c + HDL-c) (Friedewald, Levy and Fredrickon, 1972

The Friedewald equations are fairly accurate for samples in which the triglyceride concentration is less than 4.13mmol/L (Friedewald, Levy and Fredrickon, 1972).

Principle of total cholesterol assay

Cholesterol reagent is used to measure cholesterol concentration by a timed endpoint method. In the reaction, cholesterol esterase hydrolyses cholesterol esters to free cholesterol and fatty acids. Free cholesterol is oxidised to cholestene-3-one and hydrogen peroxide by cholesterol oxidase. Peroxidase catalyses the reaction of hydrogen peroxide with 4-aminoantipyrine and phenol to produce coloured quinoneimine product.

Principle of triglycerides assay

The triglyceride GPO Reagent[®] is used to measure the triglyceride concentration by a timed endpoint method. Triglycerides in the sample are hydrolysed into glycerol and free fatty acids by the action of lipase. A sequence of three coupled enzymatic steps using glycerol kinase, glycerophosphate oxidase, horseradish peroxidase causes the oxidative coupling of 3, 5 dichloro-2-hydroxybenzenesulfonic acid with 4-aminoantipyrine to form a red quinoneimine dye.

Principle of HDL-c assay

The HDL-cholesterol reagent is used to measure the cholesterol concentration by a timed endpoint method. In the reaction, the cholesterol esterase hydrolyses the cholesterol esters to free cholesterol and fatty acids. The free cholesterol is oxidised to cholesten-3-one and hydrogen peroxide by cholesterol oxidase. Peroxidase catalyses the reaction of hydrogen peroxide with 4-aminoantipyrine and phenol to produce a coloured quinoneimine product.

2.9.5 Data presentation

All data were expressed as means ± standard error of means (SEM). Data for untreated non-infected and *P. berghei* infected rats were used as baseline (control values). OGT responses to various doses of oral CHQ and pectin CHQ matrix patch as well as effects of oral and transdermal CHQ delivery methods on insulin secretion were presented separately for the non-infected and *P berghei* infected rats. For chronic studies, the calculation of mean daily fluid

voided and urinary amounts of electrolytes excreted and evaluation of GFR assessed renal function. The MAP and plasma lipid profile parameters were presented graphically for the separate groups of animals to assess the effect of the CHQ delivery routes on cardiovascular function. GraphPad InStat Software (version 5.00, GraphPad Software, San Diego, California, USA) was used for all statistical analyses. One way one-way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparison test was used to establish statistical comparison between various groups. Values of p <0.05 were taken to imply statistical significance.

CHAPTER 3

CHQ effects on blood glucose and malaria parasites

3.1 General

This chapter describes the following:

1) CHQ concentrations in the matrix patch and plasma

2) acute effects of oral CHQ treatments on OGT responses

3) short term effects oral CHQ treatments treatment on:

    i.   blood glucose and plasma insulin concentrations

    ii.   hepatic and muscle glycogen concentrations

    iii.   on malaria parasites

3.2 CHQ concentration in the matrix patch

The hydrogel matrix patch used in this study had an area of $64mm^2$ containing CHQ concentration of 15.9mg/ml translating into a dosage of 53mg/kg for a 300g rat. The percentage CHQ incorporation into the matrix patch was 74%.

3.2 1 CHQ plasma pharmacokinetics.

Figure 1 A shows plasma CHQ concentration profiles at different time intervals following oral CHQ (60mg/kg) treatment, twice daily, 8 hours apart. The peak plasma CHQ concentration was achieved after 4 hours. Figure 1B shows the profile of CHQ concentration at different time intervals following a single topical application of pectin matrix patch (53mg/kg). Peak plasma CHQ concentration was also achieved after 4 hours by comparison to oral CHQ treatment. The peak plasma CHQ concentration was sustained for 3 days after treatment with the pectin matrix patch by comparison to oral treatment where it was reduced within hours of treatment.

In the post treatment period, plasma CHQ concentrations were significantly reduced in the oral CHQ group by comparison to the patch treated group (p>0.05 (Figure 2).
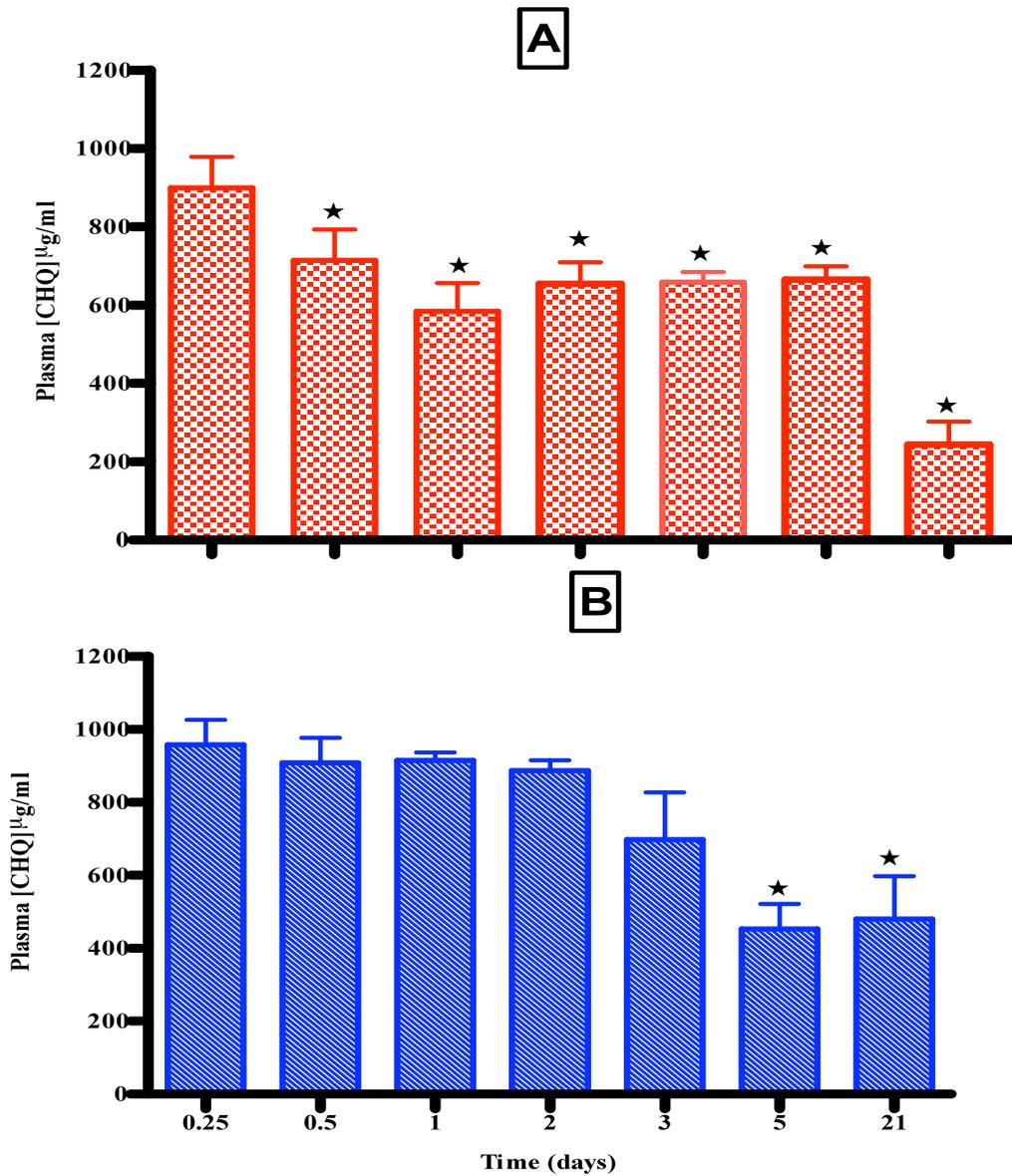
Figure 1: Effects of oral CHQ (60mg/kg) treatment **(A),** and single CHQ matrix patch (53mg/kg) **(B)** on plasma CHQ pharmacokinetics of non infected animals. Values are presented as means ± S.E.M. Vertical bars represent S.E.M. (n=6 in each group) ★ $p<0.05$ by comparison with the peak plasma CHQ concentration
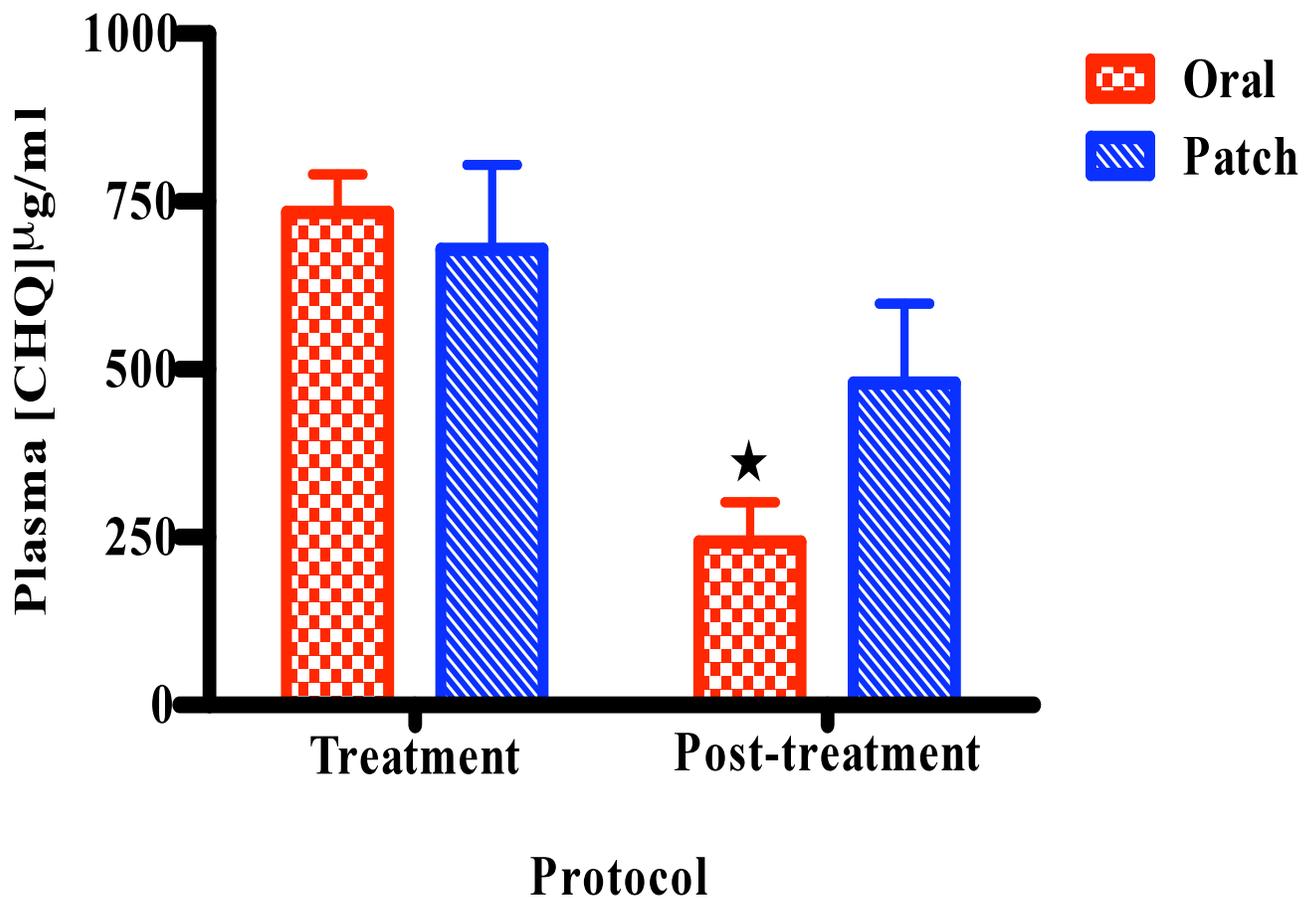
Figure 2: Plasma CHQ concentrations in animals treated twice daily with oral CHQ (60mg/kg) and those treated with a single topically applied CHQ patch (53mg/kg). Values are presented as means ± SEM where columns represent means and vertical bars represent SEM (n=6 in each group) ★ $p<0.05$ by comparison with orally treated animals.

3.3 Effects of CHQ treatments on malaria parasites

The effects of oral CHQ and pectin CHQ matrix patch treatment on parasitaemia was investigated in *P. bergei* infected animals. (Figure 3). Figure 3 shows that the peak percentage parasitaemia of control animals was reached at day 14 ($51.5 \pm 16.5\%$). The control animals were sacrificed on day 14 of the experimental period based on preliminary results. As such, all the subsequent results showing the infected control animals will be having no post treatment period (days 13-21). Following pectin CHQ matrix patch and oral CHQ treatment, the malaria parasites were cleared from systemic circulation after 3and 4 days, respectively.
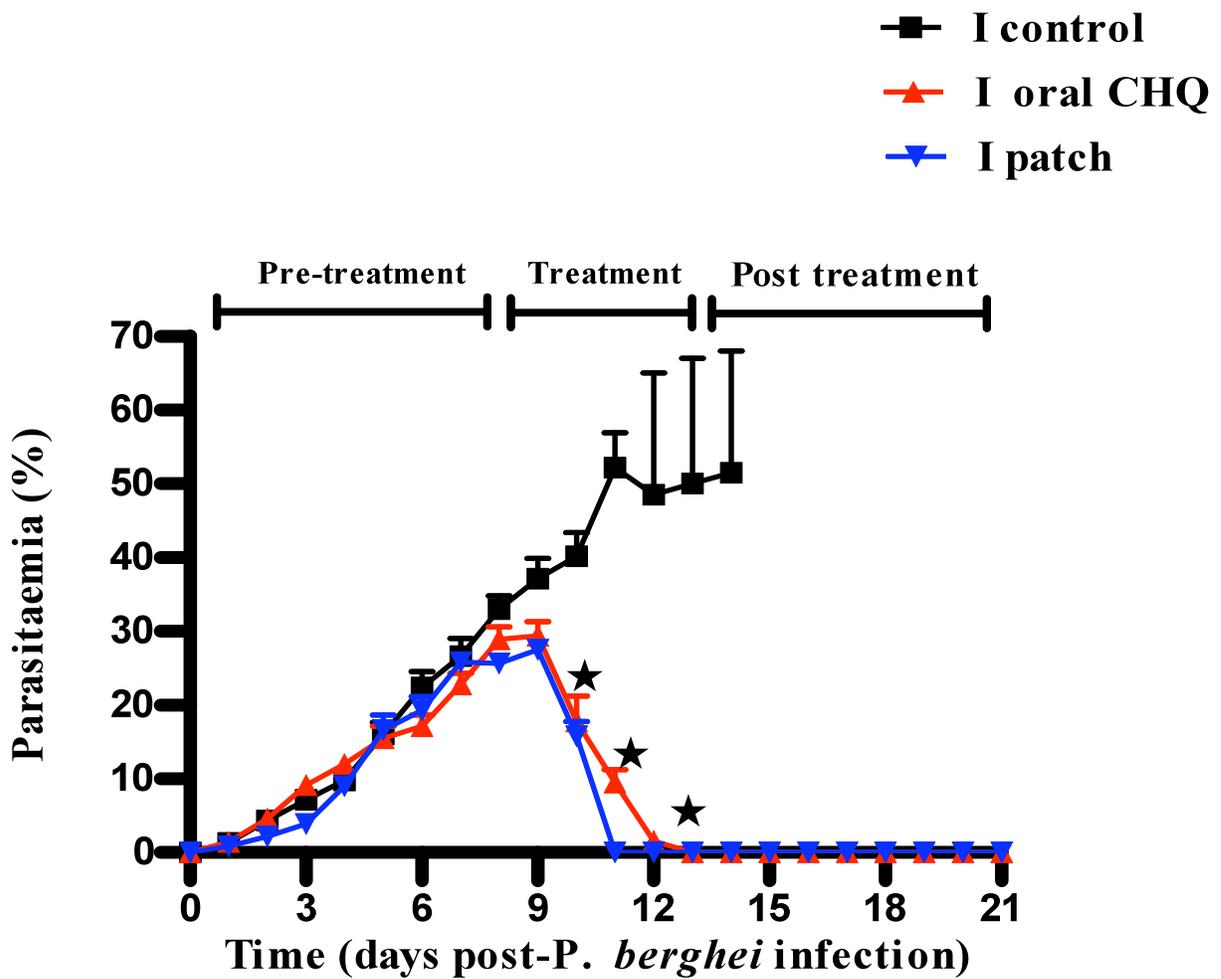
Figure 3: Percentage parasitaemia in animals treated twice daily with oral CHQ (60mg/kg) and those treated with a single topically applied CHQ patch (53mg/kg) Values are presented as means and vertical bars indicate SEM (n=6 in each group) ★ $p<0.05$ by comparison with control animals.

3.4 Acute studies

3.4.1 OGT responses

Oral glucose tolerance (OGT) responses to various doses of oral CHQ (30, 60, 120 mg/kg,) and topical application of various doses of pectin CHQ hydrogel matrix patch (29, 53, 72mg/kg) were monitored in non infected and *P. berghei* infected male Sprague- Dawley rats.

3.4.2. Effects of CHQ

Following a glucose load (0.86g/kg) in both the non infected and infected control groups, there was an increase in blood glucose concentration, from preloading values. The blood glucose concentrations of control animals steadily decreased throughout the 4 hour experimental period to concentrations, which were not significantly different from the preloading values at the end of the experimental period. All doses of CHQ administered orally (30, 60, 120mg/kg) significantly ($p<0.05$) decreased blood glucose concentrations in both non infected and infected animals in a non dose dependent manner (Figures 4 and 5). The highest dose of CHQ (120mg/kg), exhibited the most potent effect, decreasing blood glucose concentrations to hypoglycaemic values by the end of the experimental period.
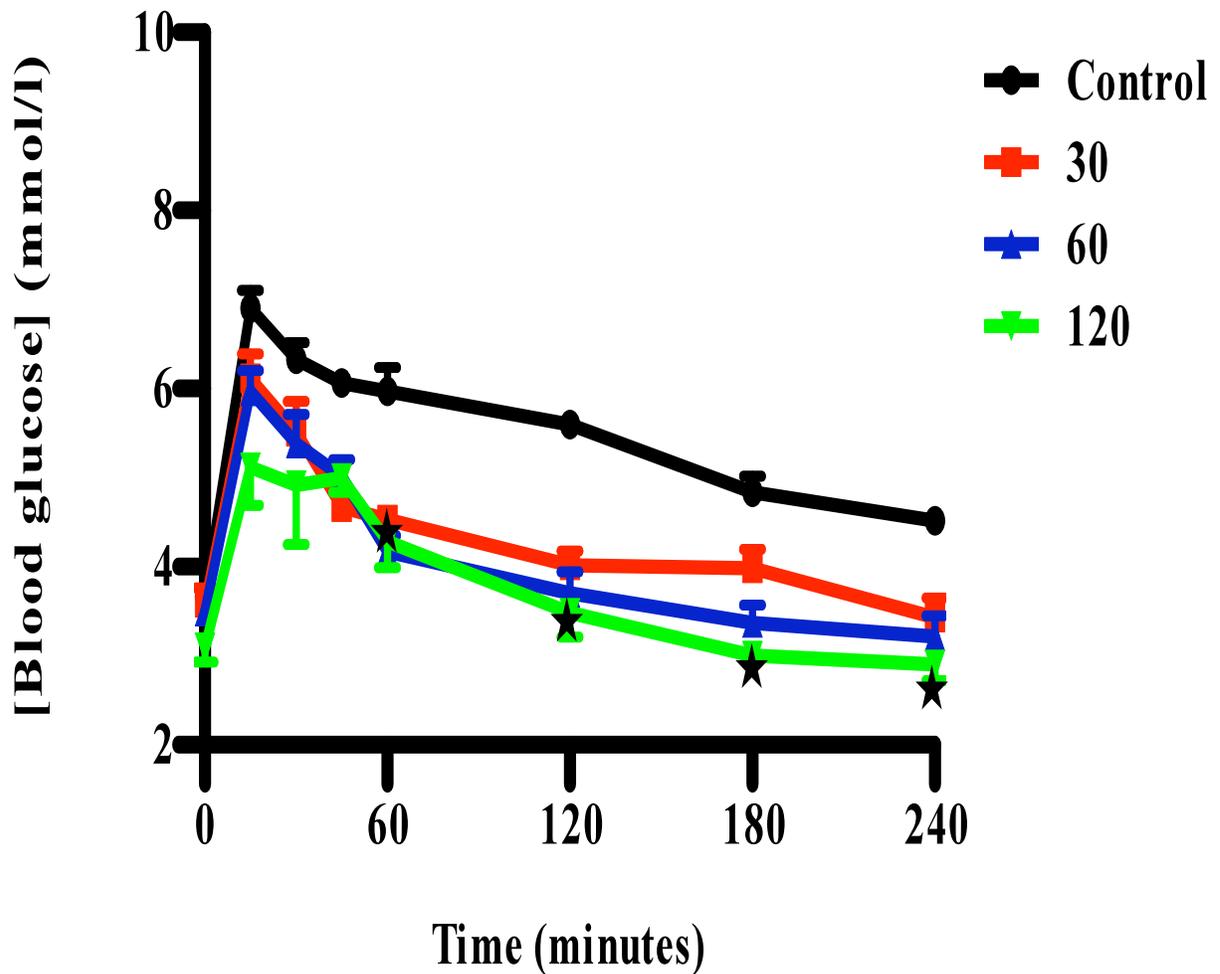
Figure 4: Comparison of OGT responses of non infected animals to various doses of oral CHQ treatment (30, 60, 120mg/kg) with respective control rats. Values are presented as means where vertical bars represent S.E.M (n=6 in each group) ★ $p < 0.05$ by comparison with control animals.
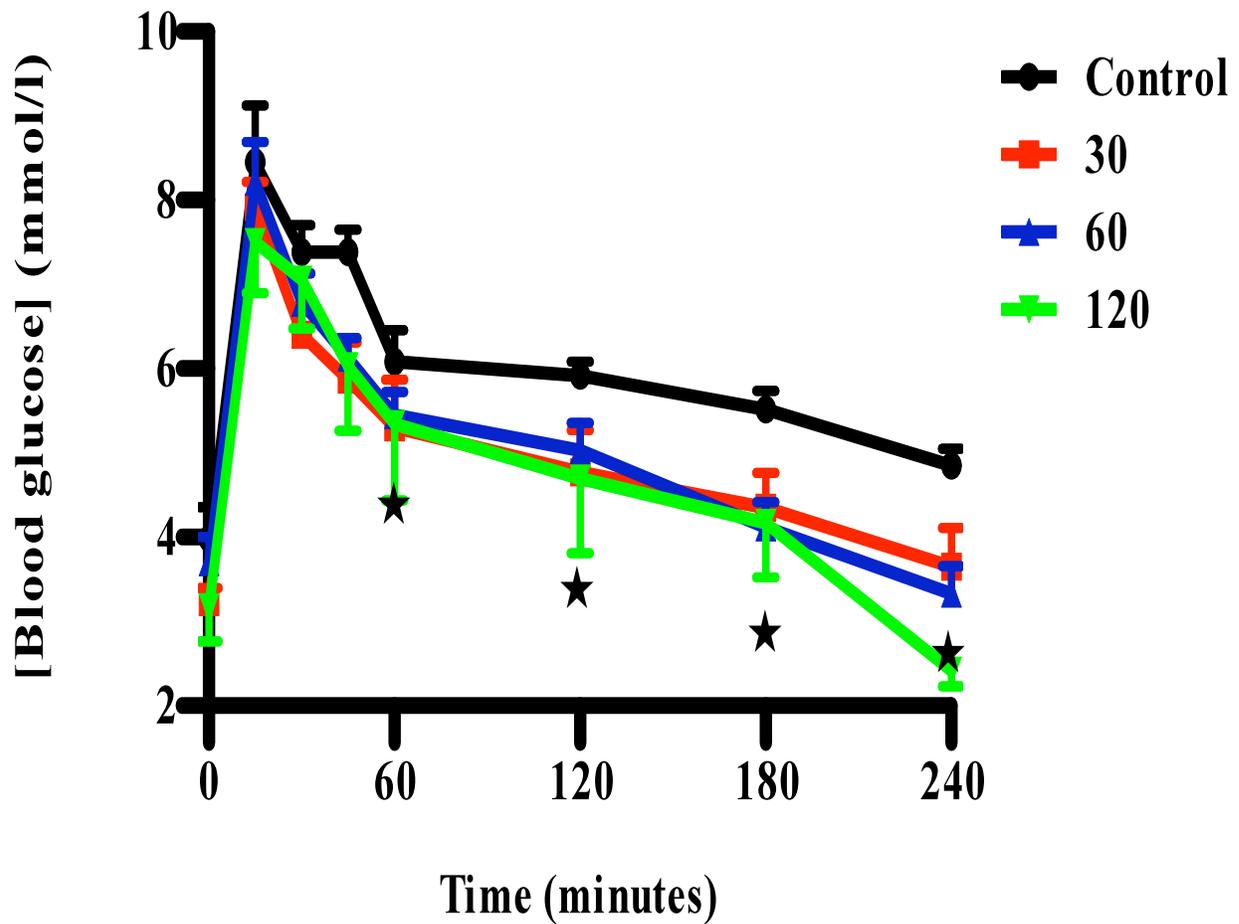
Figure 5: Comparison of OGT responses of infected animals to various doses of oral CHQ treatment (30, 60, 120mg/kg) with respective control rats. Values are presented as means where vertical bars represent SEM (n=6 in each group) ★ p<0.05 by comparison with control animals

3.4.3. Effects of pectin CHQ matrix patch

All the pectin CHQ matrix patch doses (29, 53, 72mg/kg) significantly ($p < 0.05$) decreased blood glucose concentrations in a non dose dependent manner by comparison to control (Figures 6 and 7). The highest dose of CHQ (120mg/kg), exhibited the most blood glucose lowering effect.
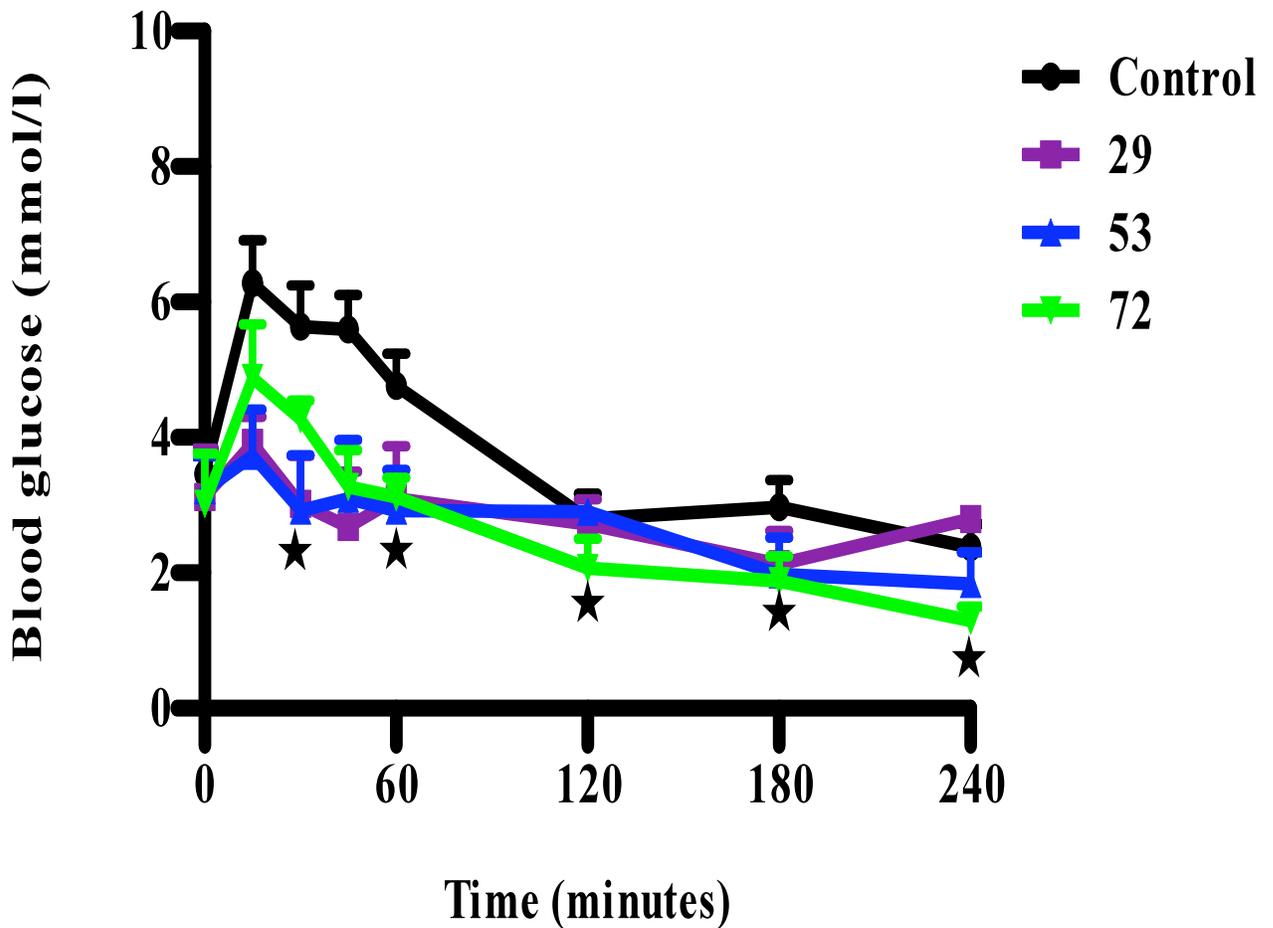
Figure 6: Comparison of OGT responses of non infected animals to various doses of transdermal pectin CHQ matrix patch treatment (29, 53, 72mg/kg) with respective control rats. Values are presented as means where vertical bars represent SEM (n=6 in each group) ★p<0.05 by comparison with control animals.
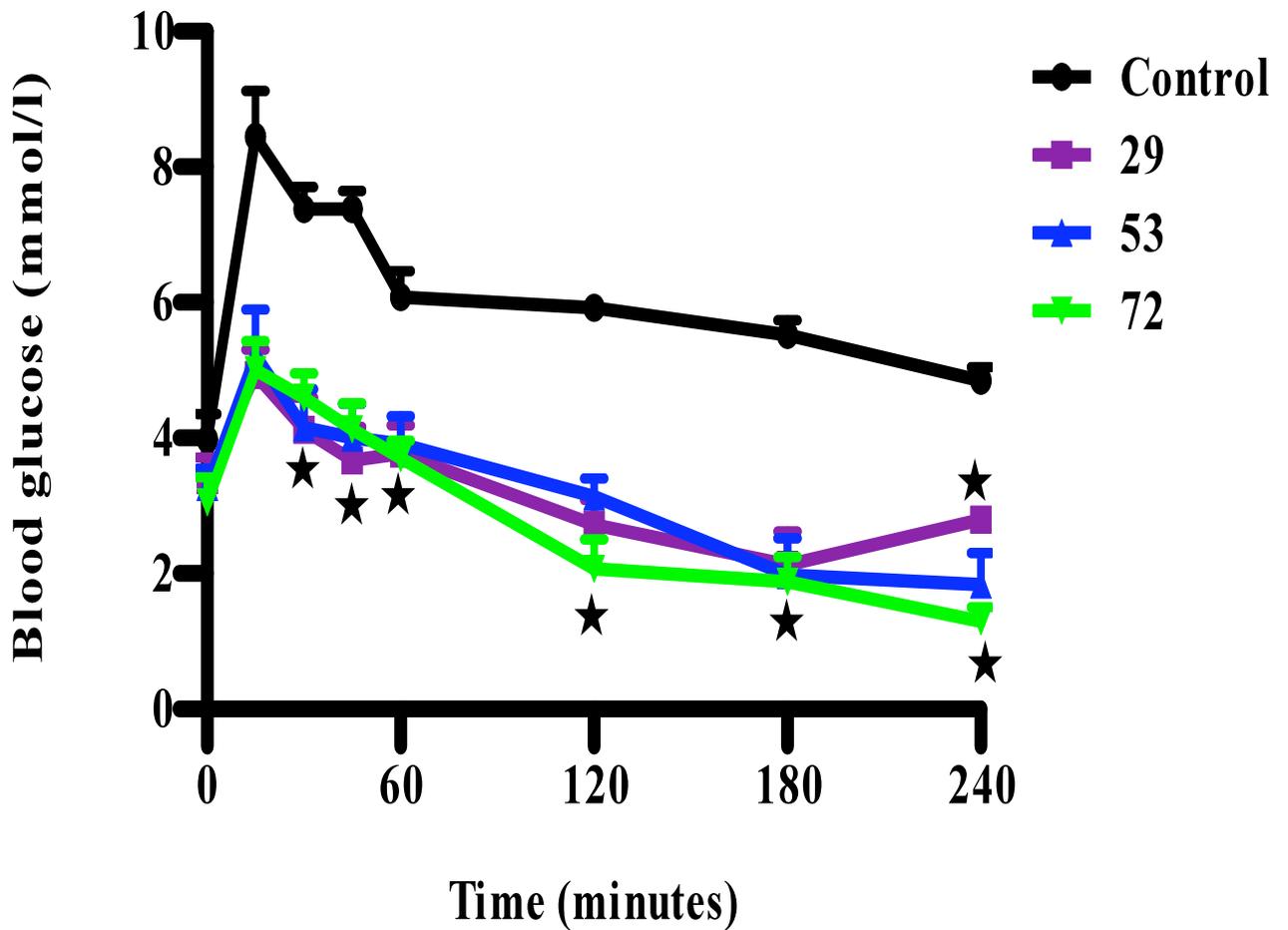
Figure 7: Comparison of OGT responses of infected animals to various doses of transdermal pectin CHQ matrix patch treatment (29, 53, 72mg/kg) with respective control rats. Values are presented as means where vertical bars represent SEM (n=6 in each group) ★p<0.05 by comparison with control animals

3.5 Short term studies

3.5.1 General

Short term studies were carried out in non infected and *P. berghei* infected male Sprague-Dawley rats over a 21 day experimental period divided into pre-treatment, treatment and post treatment periods. Blood glucose concentrations were measured every 3rd day during the pre-treatment and post treatment period, and daily during the treatment period. Separate groups of non fasted conscious animals (n=6) were sacrificed on days 0, 7, 8, 9, 10, 12, 14 and 21 to collect blood for the measurement of plasma insulin concentration and harvesting of the liver and gastrocnemius muscle tissues. The harvested tissues were used to measure hepatic and gastrocnemius muscle glycogen concentration..

3.5.2 Effects of CHQ treatments on blood glucose concentration

The effects of CHQ treatments on blood glucose concentration were investigated in separate groups of non infected and *P. berghei* infected animals treated with oral CHQ (60 mg/kg) and pectin matrix patch (53 mg/kg) in a 21 day experimental study

The blood glucose concentrations of infected control animals significantly decreased during the treatment period by comparison with non infected control animals (Figure 8).

Oral CHQ treatment, however, significantly decreased the blood glucose concentrations of both the non infected and infected animals in comparison to non infected and infected control animals (Figure 8A).

The mean blood glucose concentration of the animals treated with pectin CHQ matrix patch remained stable throughout the experiment by comparison to both non infected and infected baseline values. Treatment with pectin CHQ matrix patch did not alter the blood glucose concentration of both the non infected and infected animals by comparison to oral CHQ treated groups. (Figure 8B). In summary, *P. berghei* infection and oral CHQ treatment reduced blood glucose concentrations in male Sprague Dawley rats, while topical application of pectin CHQ matrix patch did not alter the blood glucose concentration of the animals.
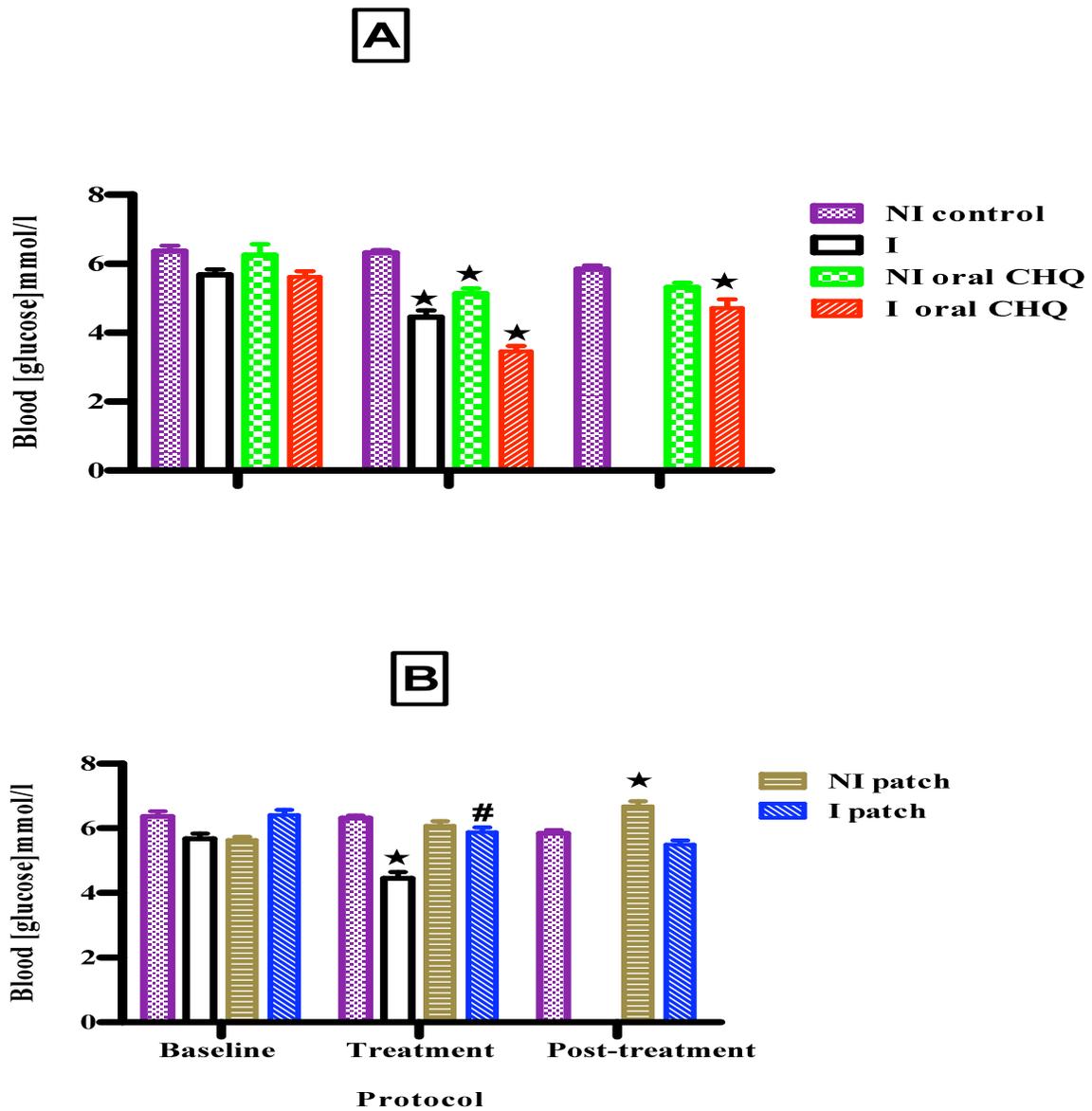
Figure 8: The effects of oral CHQ (60mg/kg) treatment (A) and single topical application of CHQ matrix patch (53mg/kg) (B) on blood glucose concentration. Values are presented as mean $\pm$ S.E.M, where columns represent means and vertical bars represent SEM (n=6 in each group). ★p<0.05 by comparison to baseline control animals. # p<0.05 by comparison with oral CHQ treated animals.

3.5.2 Effects of CHQ treatments on plasma insulin concentration

Effects of twice daily oral CHQ (60mg/kg) and a once off application of pectin CHQ matrix patch (53mg/kg b.wt) treatment on blood glucose and plasma insulin concentrations in non infected animals were investigated in a 21 day study. Separate groups of non fasted conscious animals (n=6) were sacrificed on days 0, 7, 8, 9, 10, 12, 14 and 21 to collect blood for the measurement of plasma insulin concentration. Plasma insulin concentration of *P. berghei* infected animals was not measured in the current study.

Oral CHQ administration increased plasma insulin concentration whilst topical CHQ patch did not have any significant effect (Figure 9).
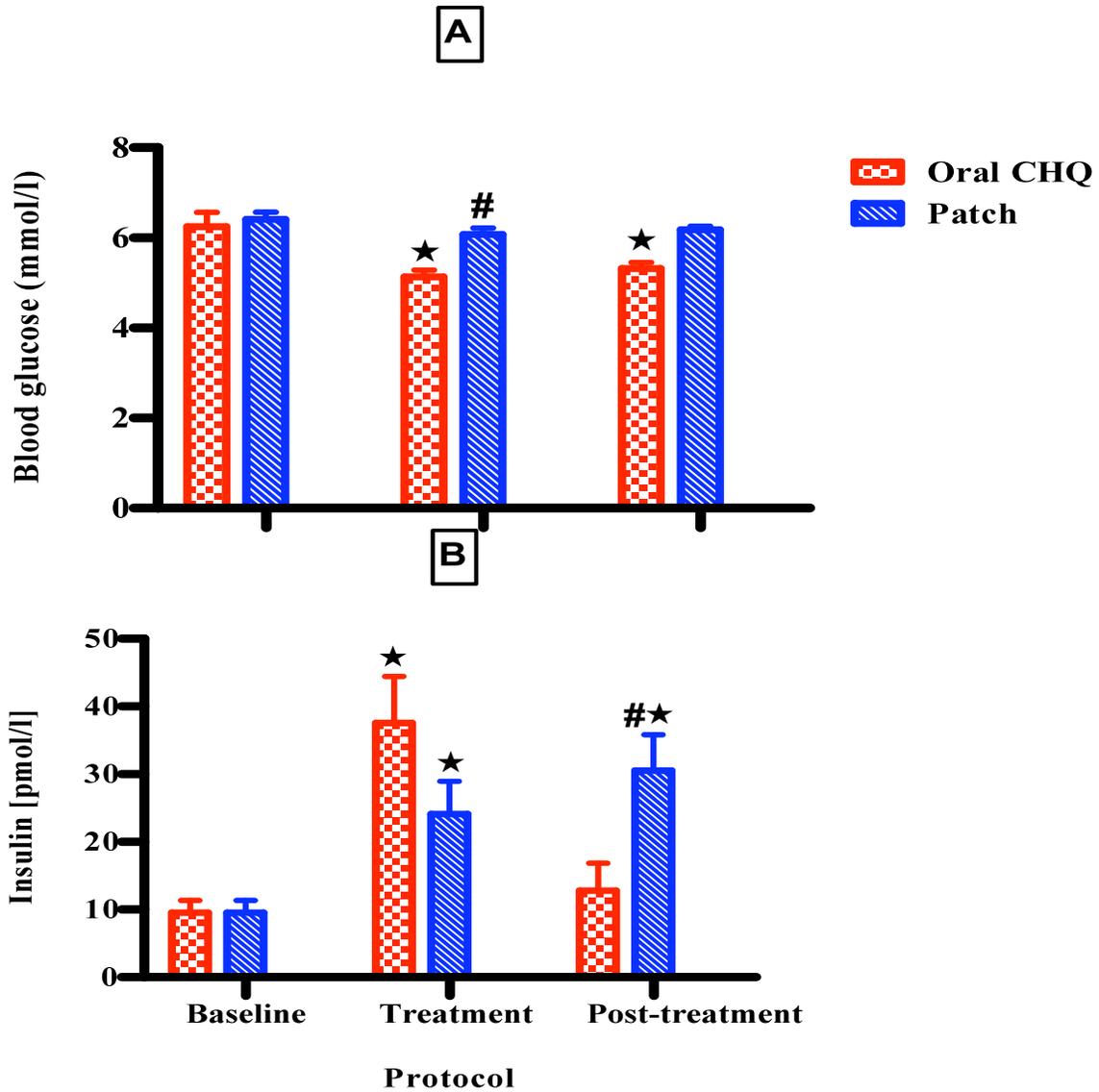
Figure 9: The effects of oral CHQ (60mg/kg) treatment and single topical application of CHQ matrix patch (53mg/kg) on blood glucose **(A)** and plasma insulin **(B)** concentrations. Values are presented as mean ± S.E.M, where columns represent means and vertical bars represent SEM (n=6 in each group). ★ $p < 0.05$ by comparison with topically applied CHQ patch treated animals. # $p < 0.05$ by comparison with oral post-treatment animals.

3.6 **Hepatic and gastrocnemius muscle glycogen concentrations**

Effects of twice daily oral CHQ (60mg/kg) and a once off application of pectin CHQ matrix patch (53mg/kg) treatment on hepatic and gastrocnemius muscle glycogen concentrations in non infected and *P. berghei* infected animals were investigated in a 21 day study. Separate groups of non fasted conscious animals (n=6) were sacrificed on days 0, 7, 8, 9, 10, 12, 14 and 21 to harvest the liver and gastrocnemius muscle tissues for the measurement of hepatic and gastrocnemius muscle glycogen concentrations.

3.6.1 Hepatic glycogen concentration

3.6.1.1 Effects of CHQ treatments on hepatic glycogen concentration

Mean hepatic glycogen concentration of both non infected and *P. berghei* infected control animals remained unchanged throughout the study period (Figure 10A). Oral CHQ treatment significantly increased hepatic glycogen concentration of non infected group by comparison to control animals (Figure 10A). The mean hepatic glycogen concentration of the animals treated with pectin CHQ matrix patch remained stable throughout the experimental period by comparison to the non infected control and oral CHQ groups (Figure 10B).

3.6.2 Gastrocnemius muscle glycogen concentration

3.6.2.1 Effects of CHQ treatments on gastrocnemius muscle glycogen concentration

Mean gastrocnemius muscle glycogen concentration of both the non infected and *P. berghei* infected control animals did not significantly differ throughout the experimental period. Oral CHQ treatment significantly decreased gastrocnemius muscle glycogen concentration of the non infected group, during the post treatment period in comparison to baseline and treatment periods. Topical application of pectin CHQ patch decreased the gastrocnemius muscle glycogen concentration of the infected group, during the post treatment period in comparison to baseline and treatment periods.
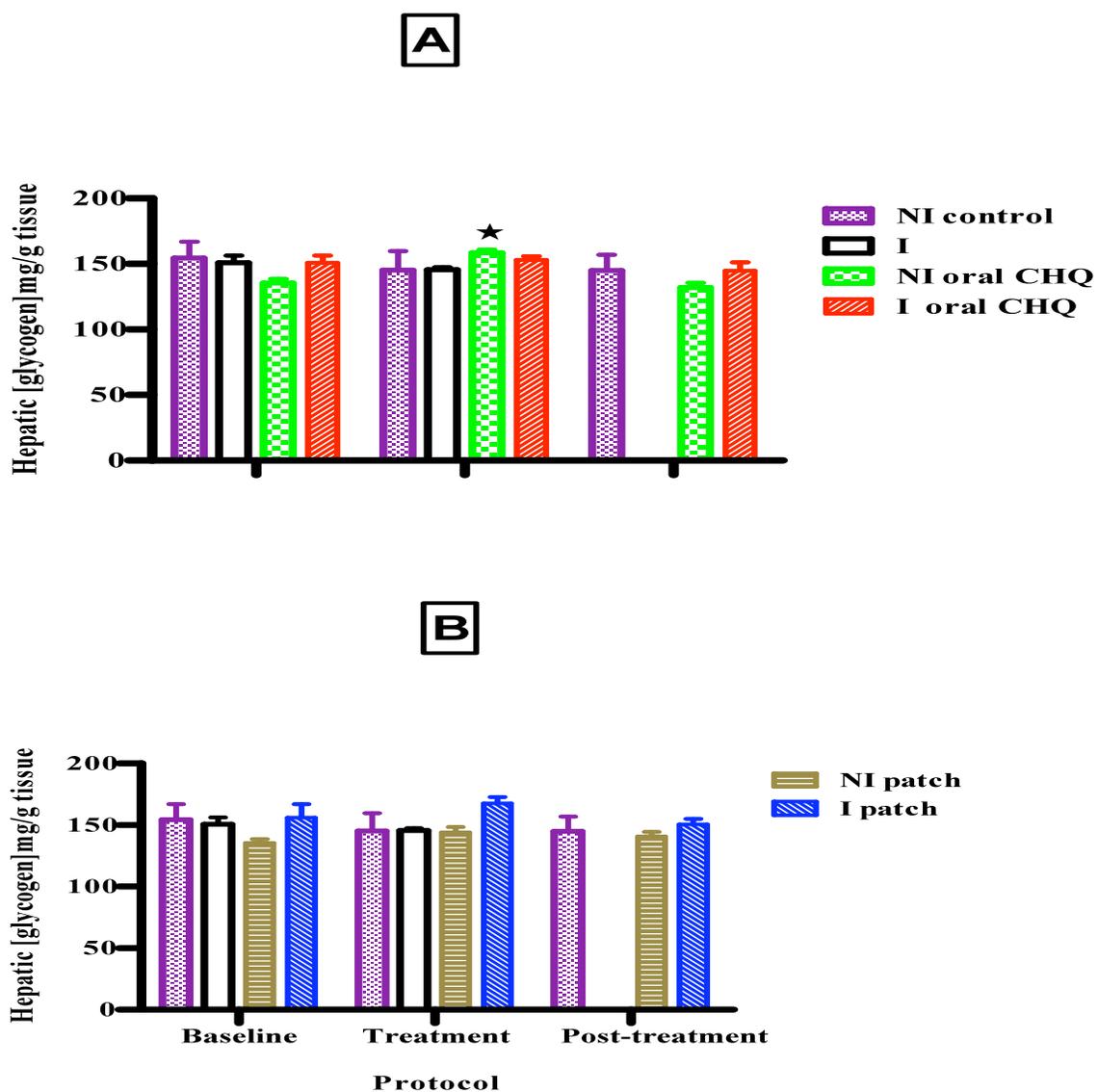
Figure 10: The effects of oral CHQ (60mg/kg) treatment **(A)** and single topical application of CHQ matrix patch (53mg/kg) **(B)** on hepatic glycogen concentration. Values are presented as mean ± S.E.M, where columns represent means and vertical bars represent SEM (n=6 in each group). ★ p<0.05 by comparison with baseline control animals.
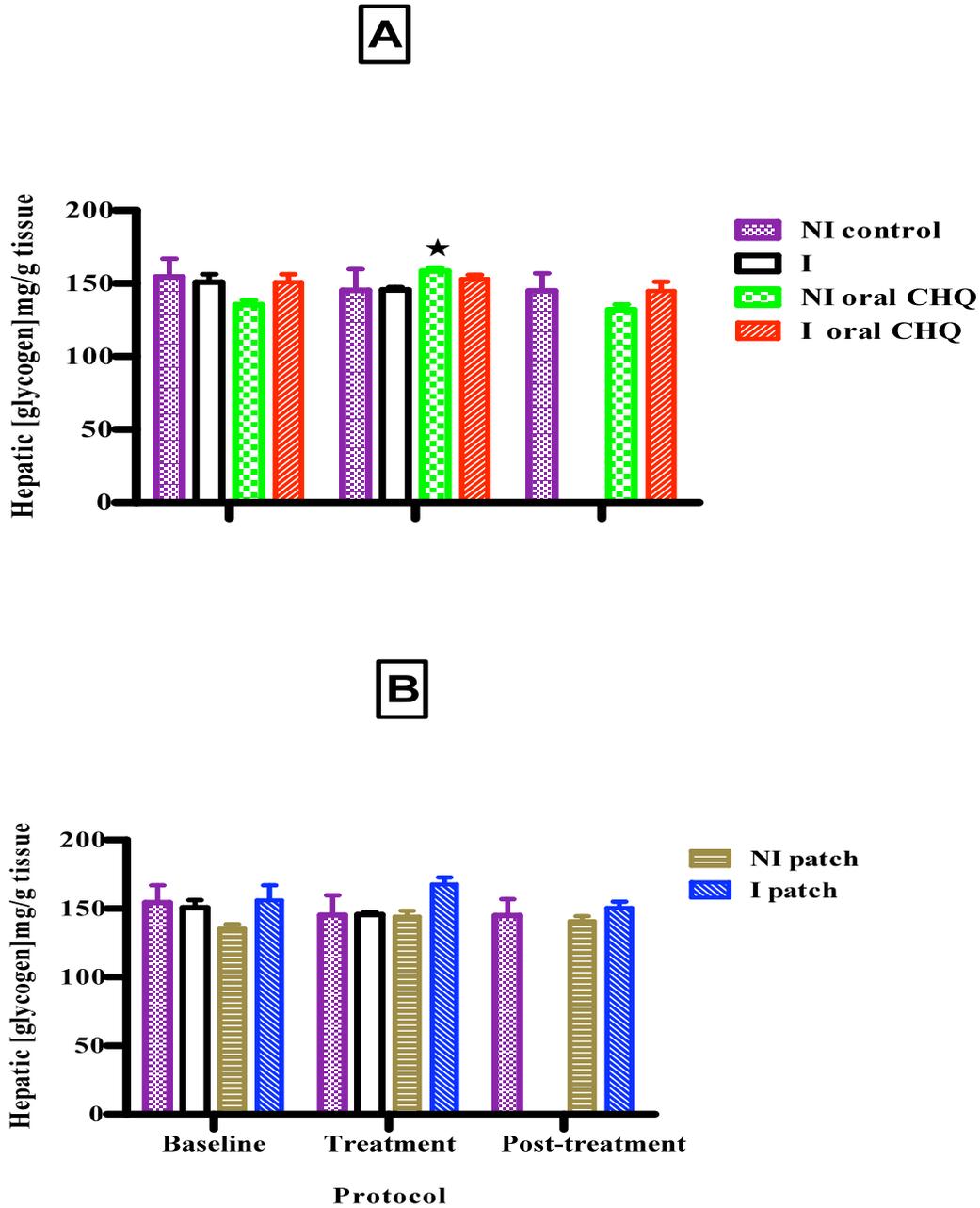
Figure 11: The effects of oral CHQ (60mg/kg) treatment **(A)** and single topical application of CHQ matrix patch (53mg/kg) **(B)** on gastrocnemius muscle glycogen concentration. Values are presented as mean ± S.E.M, where columns represent means and vertical bars represent SEM (n=6 in each group). ★p<0.05 by comparison with baseline control animals.

3.7 Hepatic and gastrocnemius muscle mass

The hepatic and gastrocnemius muscle mass of non infected control animals did not differ significantly throughout the experimental period. There was a significant increase in hepatic mass of infected control animals and the infected group of animals that were treated with oral CHQ or pectin CHQ matrix patch. The hepatic mass of non infected animals treated with oral CHQ and pectin matrix patch did not differ significantly throughout the experimental period.

There were no significant differences in the muscle mass of both non infected and infected control animals, as well as the treated groups as shown in Table 1.

Table 1: Hepatic and gastrocnemius muscle masses in separate groups of non infected and infected groups of animals treated twice daily with oral CHQ and pectin CHQ matrix patch, for baseline/pre-treatment (day 0-7), treatment (day 8-12) and post-treatment (day 13-21) periods. ★$p<0.05$ in comparison to non infected control animals (n=6 in each group).

Organ (g/100g tissue)	Protocol		Pre-treatment	Treatment	Post-treatment
Hepatic mass	**Control**	Non infected	3.93 ± 0.08	3.81 ± 0.08	3.94 ± 0.06
		P. berghei infected	6.74 ± 0.30	8.65 ± 0.43★	N/A
	Oral	Non infected	3.93 ± 0.08	4.24 ± 0.12	4.46 ± 0.88
		P. berghei infected	6.44 ± 0.16	7.07 ± 0.17★	4.39 ± 0.14
	Patch	Non infected	4.11 ± 0.24	4.29 ± 0.09	3.90 ± 0.11
		P. berghei infected	6.44 ± 0.16	6.38 ± 0.48★	5.41 ± 0.30
Muscle mass	**Control**	Non infected	1.13 ± 0.11	1.72 ± 0.11	1.82 ± 0.14
		P. berghei infected	0.94 ± 0.04	1.00 ± 0.09	N/A
	Oral	Non infected	1.13 ± 0.11	1.38 ± 0.07	2.08 ± 0.05
		P. berghei infected	0.99 ± 0.88	1.15 ± 0.04	1.37 ± 0.06
	Patch	Non infected	1.79 ± 0.10	1.45 ± 0.08	1.85 ± 0.12
		P. berghei infected	0.99 ± 0.66	1.12 ± 0.08	1.14 ± 0.08

Not available (N/A) as the infected control animals were sacrificed on day 14 based on the preliminary study findings.

Chapter 4
CHQ effects on renal fluid and electrolyte handling

4.1 General

This chapter describes the effects of CHQ treatments on:

i.   body weight, food and water intake

ii.   renal fluid and electrolyte handling

4.2 Effects of CHQ on body weight, food and water intake

The food intake of the non infected control animals did not differ significantly throughout the pre treatment, treatment and post treatment periods. However, oral CHQ administration to the non infected and infected groups of animals reduced food intake by comparison with animals on which the CHQ patch was applied onto the shaved skin area on the back of the neck skin (Table 2).

Water intake of the non infected, infected control animals and those rats topically applied the pectin matrix patch did not differ significantly throughout the experimental period whilst water intake decreased in infected animals that were treated with oral CHQ (Table 2).

The percentage body weight change of the non infected control animals did not differ significantly throughout the experimental period. There was, however, a significant decrease in the percentage body weight change of infected animals during the treatment period by comparison to the non infected control animals. The percentage body weight changes of both the non infected and infected group of animals treated twice daily with oral CHQ, decreased significantly by comparison to the non infected control animals and with the pectin CHQ matrix patch treated group of animals (Table 2).

Table 2: Effects of twice daily with oral CHQ (60mg/kg) treatment and a single topically applied pectin CHQ matrix patch (53mg/kg) on food intake, water intake and body weight changes in non infected and *P. berghei* infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group) ★p<0.05 by comparison with control animals. # p<0.05 by comparison with oral CHQ treated animals

| Parameter	Protocol		Pre-treatment	Treatment	Post-treatment
Food intake (g/day)	**Control**	Non infected	18 ± 0.3	19 ± 1	17 ± 1
		P. berghei infected	14 ± 0.2	10 ± 1.0	N/A
	Oral	Non infected	18 ± 0.3	13 ± 0.4*#	23 ± 1
		P. berghei infected	15 ± 0.3	11 ± 1*#	19 ± 2
	Patch	Non infected	18 ± 0.3	18 ± 0.2	21 ± 1
		P. berghei infected	16 ± 1	16 ± 1	27 ± 1
Water intake (ml/day)	**Control**	Non infected	25 ± 1	22 ± 1	18 ± 1
		P. berghei infected	21 ± 1	18 ± 1	N/A
	Oral	Non infected	21 ± 1	20 ± 1	26 ± 1
		P. berghei infected	20 ± 1	12 ± 1*#	22 ± 2
	Patch	Non infected	25 ± 1	23 ± 0.4	26 ± 1
		P. berghei infected	20 ± 1	21 ± 1	30 ± 1
% b.wt change	**Control**	Non infected	32 ± 3	31 ± 1	31 ± 1
		P. berghei infected	40 ± 5	-18 ± 1*	N/A
	Oral	Non infected	34 ± 2	16 ± 0.4*#	31 ± 1
		P. berghei infected	37 ± 4	-5 ± 3*#	47 ± 7
	Patch	Non infected	28 ± 4	44 ± 1	24 ± 1
		P. berghei infected	16 ± 4	2 ± 7*	60 ± 13

N/A as the infected control animals were sacrificed on day 14 based on the preliminary study findings.

4.3 Effects of CHQ on renal fluid and electrolyte handling

The results are presented according to the experimental protocol, which was divided into pre treatment (baseline, 0-7days), treatment (8-12 days) and post treatment period (13-21 days).

The 24 hour urine output of non infected animals was stable throughout the 21 day experimental period. *P. berghei* infected animals had significantly lower 24 hour urine outputs by comparison to non infected control animals (Figure 12). Oral CHQ and pectin CHQ matrix patch treatment significantly decreased the urine output of both the non infected and *P. berghei* infected animals by comparison to non infected control rats.

The 24 hour urinary Na^+ outputs of non infected and infected control animals remained stable throughout the 21 day experimental period. Treatment of non infected animals and infected animals with oral CHQ significantly increased the 24 hour urinary Na^+ outputs by comparison to baseline and non infected control animals (Figure 13). There were no significant differences in the urinary Na^+ outputs of animals treated with pectin CHQ matrix patch throughout the experimental period.

The non infected control animals had stable 24 hour urinary K^+ outputs throughout the experimental period. There was a significant increase in the urinary K^+ outputs of the infected animals during the treatment period by comparison to the non infected control animals. Treatment of both non infected and *P. berghei* infected animals with oral CHQ significantly increased the urinary K^+ outputs by comparison to the non infected control animals. There were no significant differences in the urinary K^+ outputs of animals treated with pectin CHQ matrix patch throughout the experimental period (Figure 14).

The urinary creatinine outputs of non infected control animals were stable throughout the 21 day experimental period. The infected control animals had significantly increased urinary creatinine outputs during the treatment period by comparison to non infected control animals. Treatment of non infected animals and infected animals with oral CHQ significantly increased the 24 hour urinary creatinine outputs by comparison (Figure 15). Urinary creatinine outputs of non infected animals treated with pectin CHQ matrix patch did not differ significantly throughout the

experimental period. The infected animals treated with pectin matrix patch had significantly higher urinary creatinine outputs by comparison to non infected control animals.

Urinary urea outputs of both the non infected and infected control animals did not differ significantly throughout the experimental period. Treatment of both infected and non infected separate groups of animals with oral CHQ or pectin CHQ matrix patch had no significant effects on urinary urea outputs (Figure 16).

In summary the results of the current study have demonstrated malaria infection and oral CHQ induced natriuresis with a decrease in urine output. The study has also demonstrated malaria oral CHQ induced hyperkalaemia and kaliuresis in malaria infected animals. Malaria infection and oral CHQ treatment elevated urinary creatinine outputs. Topical application of pectin CHQ matrix patch circumvented natriuresis, hyperkalaemia, kaliuresis and elevation of urinary creatinine outputs.
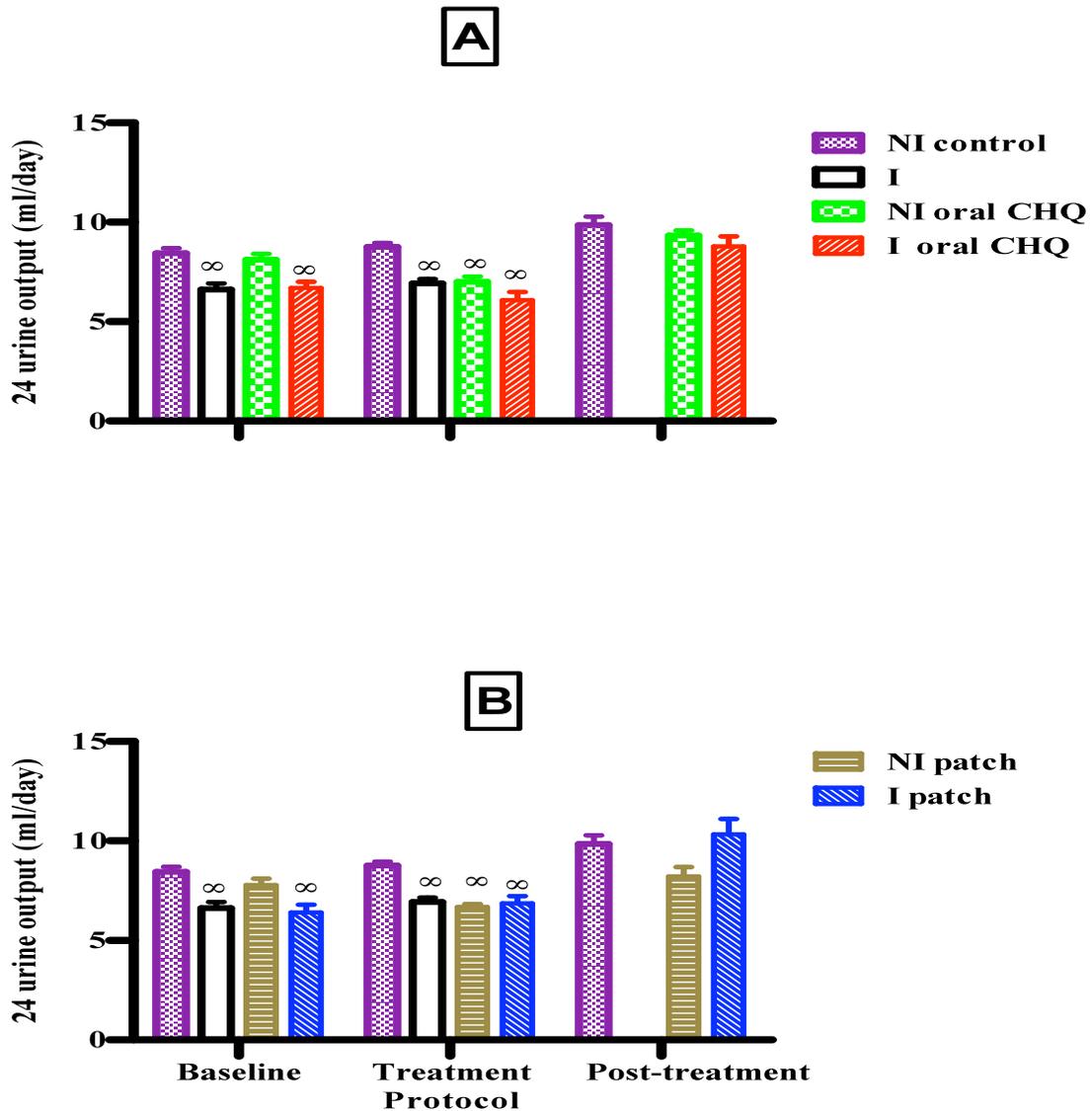
Figure 12: Comparison of the effects of twice daily treatment with oral CHQ (60mg/kg) **(A)** and with a single topically applied pectin CHQ matrix patch (53mg/kg) **(B)** on 24 hour urine output in non infected and *P. berghei* infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group) ∞p<0.05 by comparison with non infected control animals
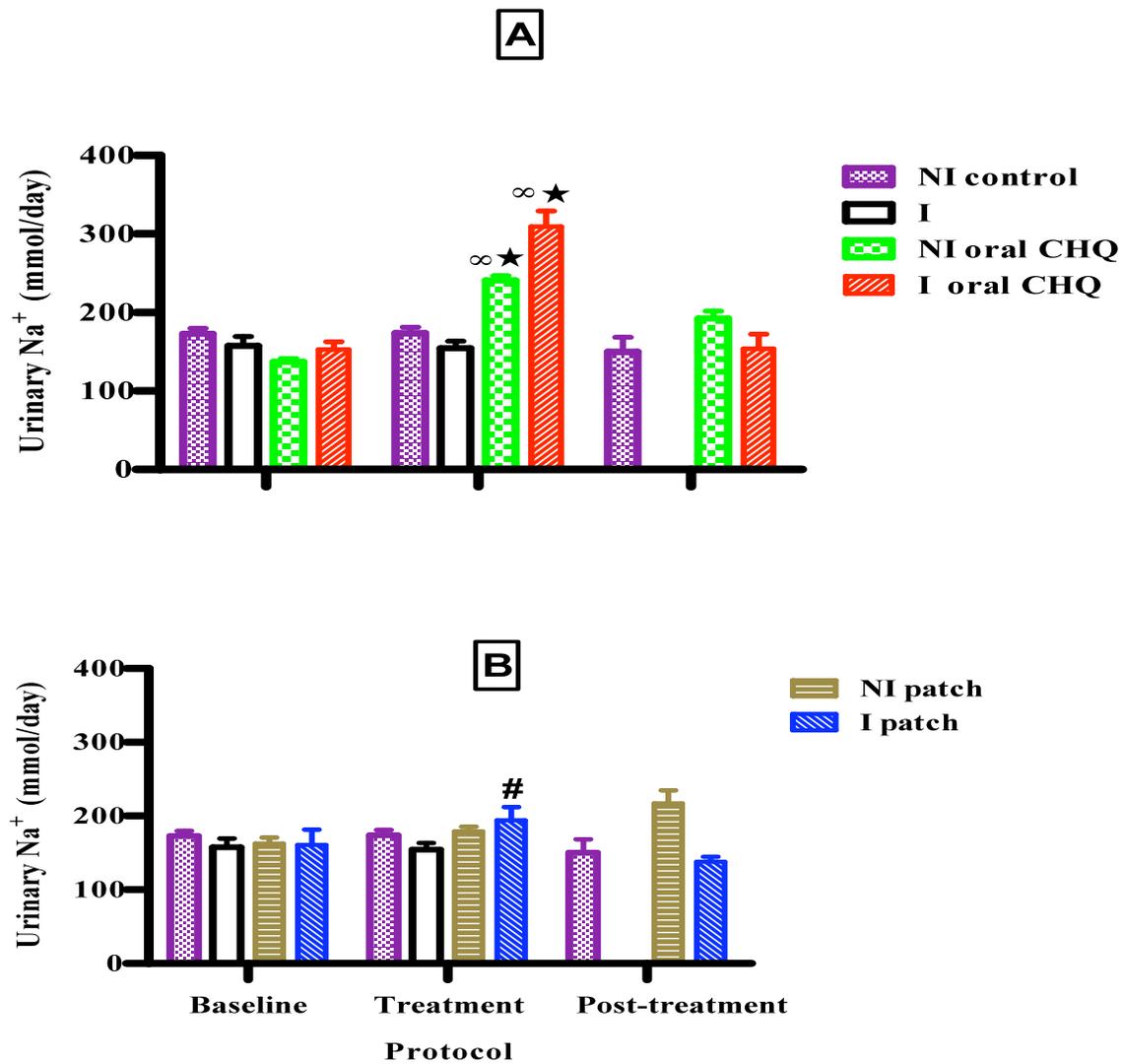
Figure 13: Comparison of the effects of twice daily treatment with oral CHQ (60mg/kg) **(A)** and with a single topically applied pectin CHQ matrix patch (53mg/kg) **(B)** on 24h urinary Na^+ outputs in non infected and *P. berghei* infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group) ★$p < 0.05$ by comparison with baseline control animals. ∞$p < 0.05$ by comparison with non infected control animals # $p < 0.05$ by comparison with oral CHQ treated animals

Figure 14: Comparison of the effects of twice daily treatment with oral CHQ (60mg/kg) **(A)** and with a single topically applied pectin CHQ matrix patch (53mg/kg) **(B)** on 24h urinary K^+ outputs in non infected and *P. berghei* infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group) ★p<0.05 by comparison with baseline control animals. ∞p<0.05 by comparison with non infected .control animals # p<0.05 by comparison with oral CHQ treated animals

Figure 15: Comparison of the effects of twice daily treatment with oral CHQ (60mg/kg) **(A)** and with a single topically applied pectin CHQ matrix patch (53mg/kg) **(B)** on 24h urinary creatinine outputs in non infected and *P. berghei* infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group) ★$p<0.05$ by comparison with baseline control animals. ∞$p<0.05$ by comparison with non infected .control animals # $p<0.05$ by comparison with oral CHQ treated animals
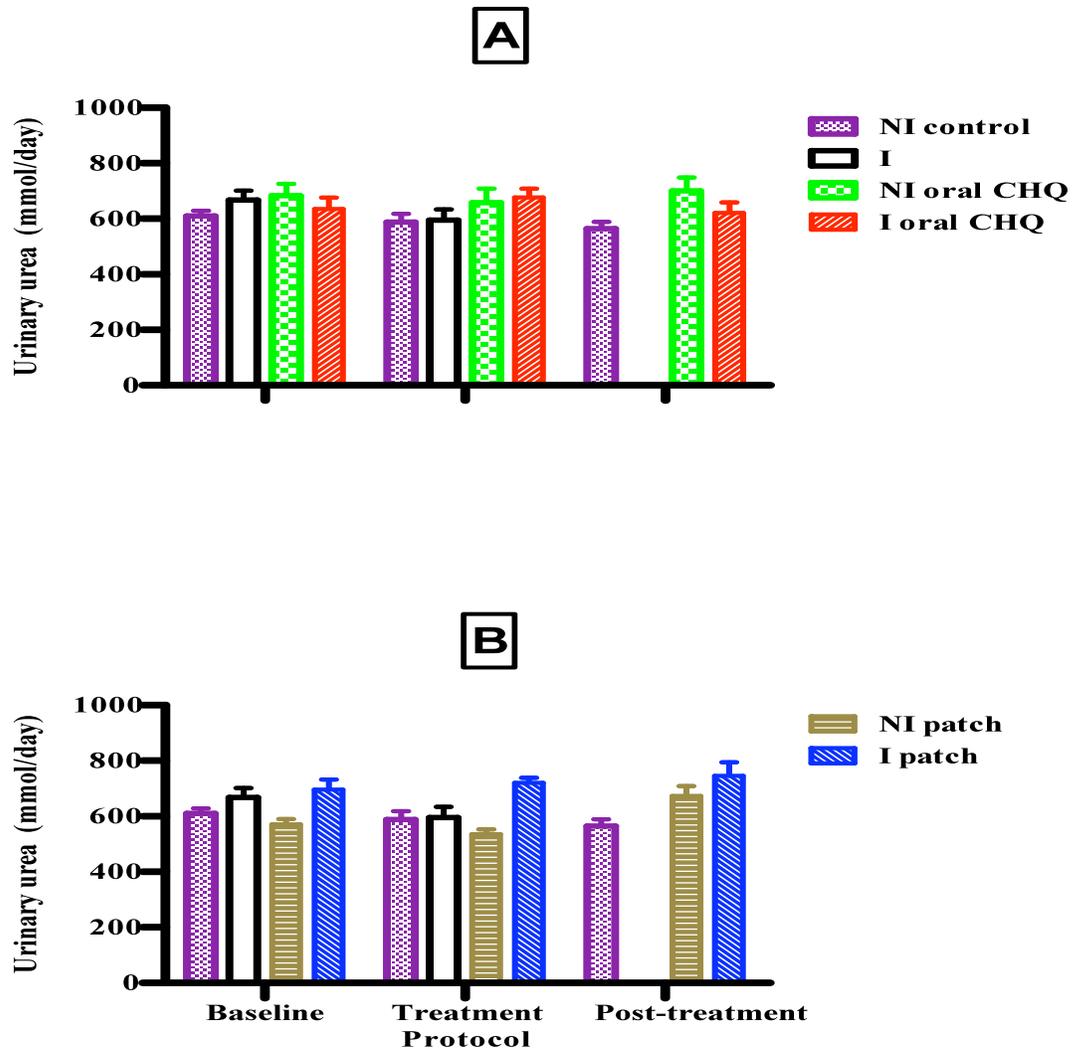
Figure 16: Comparison of the effects of twice daily treatment with oral CHQ (60mg/kg) **(A)** and with a single topically applied pectin CHQ matrix patch (53mg/kg) **(B)** on 24h urinary urea outputs in non infected and *P. berghei* infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group)

4.4 Effects of CHQ on plasma biochemical parameters

Table 3 shows the effects of treatment twice daily with oral CHQ (60mg/kg) or with a single topically applied pectin CHQ matrix patch (53mg/kg) on plasma biochemical parameters in non infected and *P. berghei* infected animals

All the plasma biochemical parameters of infected control animals did not differ significantly throughout the experimental study. The plasma K^+ concentrations of the *P. berghei* infected group of animals treated twice daily with oral CHQ significantly increased during the treatment and post treatment periods in comparison to both the non infected and *berghei* infected control animals and when compared with pectin CHQ matrix patch treated animals (Table 3)

Table 3: Comparison of the effects of treatment twice daily with oral CHQ (60mg/kg) or with a single topically applied pectin CHQ matrix patch (53mg/kg) on plasma biochemical parameters in non infected and *P. berghei* infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group) ★p<0.05 by comparison with baseline control animals. # p<0.05 by comparison with patch treated animals

| Protocol	Parameter	Control		Oral CHQ		Patch	
		Non infected	*P. berghei* infected	Non infected	*P. berghei* infected	Non infected	*P. berghei* infected
Pre-treatment	Na$^+$ (mmol/l)	137 ± 2	130 ± 4	145 ± 2	134 ± 6	135 ± 2	135 ± 3
	K$^+$ (mmol/l)	5.89 ± 1.00	5.09 ± 0.33	4.26 ± 0.72	5.09 ± 0.33?	5.56 ± 0.31	5.49 ± 0.44
	Urea (mmol/l)	4.5 ± 0.4	32.3 ± 4.0	30.7 ± 1.0	30.6 ± 0.9	30.7 ± 1.0	31.0 ± 1.3
	Creatinine (μmol/l)	26 ± 2	29 ± 1	22 ± 1	28 ± 1	31 ± 1	30 ± 2
	GFR (ml/min)	0.9	1.0	0.9	0.7	0.7	0.6
	Kidney mass (g/100g b.wt)	0.82 ± 0.02	1.01 ± 0.03	0.92 ± 0.03	0.83 ± 0.02	0.82 ± 0.02	0.83 ± 0.02
Treatment	Na$^+$ (mmol/l)	130 ± 5	139 ± 2	136 ± 3	139 ± 3	143 ± 1	142 ± 1
	K$^+$ (mmol/l)	6.51 ± 0.31	4.57 ± 0.88	4.73 ± 0.36	9.96 ±1.86[★#]	5.38 ± 0.41	5.38 ± 0.41
	Urea (mmol/l)	2.9 ± 0.8	29.0 ± 0.5	27.6 ± 0.8	27.6 ± 0.8	27.6 ± 0.8	23.6 ± 1.2
	Creatinine (μmol/l)	23 ± 3	30 ± 4	23 ± 3	27 ± 0.4	24 ± 1	27 ± 4
	GFR (ml/min)	1.0	1.0	0.9	0.9	0.9	0.9
	Kidney mass (g/100g b.wt)	0.88 ± 0.04	1.21 ± 0.29	0.88 ± 0.02	0.96 ± 0.02	0.90 ± 0.01	1.05 ± 0.03
Post-treatment	Na$^+$ (mmol/l)	133 ± 4	N/A	135 ± 3	138 ± 2	145 ± 1	144
	K$^+$ (mmol/l)	5.41 ± 0.16	N/A	3.73 ± 0.25	13.09 ± 3.06[★#]	5.37 ± 0.79	5.37 ± 0.79
	Urea (mmol/l)	4.2 ± 0.7	N/A	25.6 ± 1.3	25.6 ± 1.2	25.6 ± 1.2	24.0 ± 1.3
	Creatinine (μmol/l)	23 ± 3	N/A	22 ± 1	25 ± 1	24 ± 1	21 ± 1
	GFR (ml/min)	1.1	N/A	1.5	1.5	1.1	1.8
	Kidney mass (g/100g b.wt)	0.89 ± 0.02	N/A	0.91 ± 0.02	0.87 ± 0.03	0.84 ± 0.02	1.03 ± 0.05

N/A as the infected control animals were sacrificed on day 14 based on the preliminary study findings.

Chapter 5

Effects of CHQ on cardiovascular function and lipid profile

This chapter describes the effects of CHQ treatments on:

   i.   haematocrit

  ii.   mean arterial pressure

 iii.   lipid profile parameters

5.1 Haematocrit

The haematocrit of non infected animals was stable throughout the 21 day experimental period. *P. berghei* infection, however, significantly reduced haematocrit by comparison to non infected control animals. Treatment of both non infected and infected animals with oral CHQ significantly decreased haematocrit by comparison to non infected control animals (Figure 17).Topical application of pectin CHQ patch did not significantly alter the haematocrit of the non infected animals. The haematocrit of the *P. berghei* infected group of animals treated with pectin CHQ patch was significantly reduced in comparison to non infected control animals.
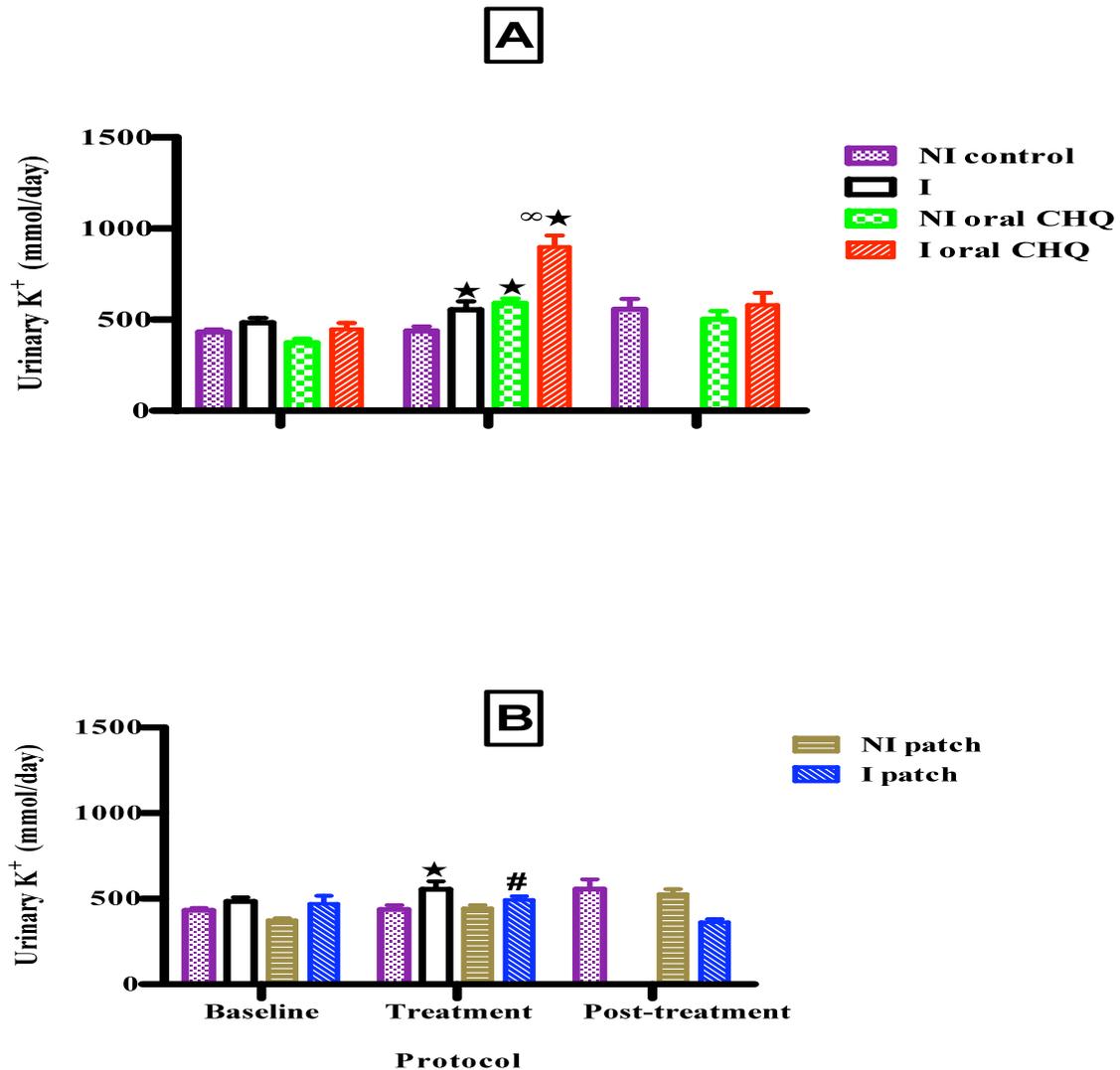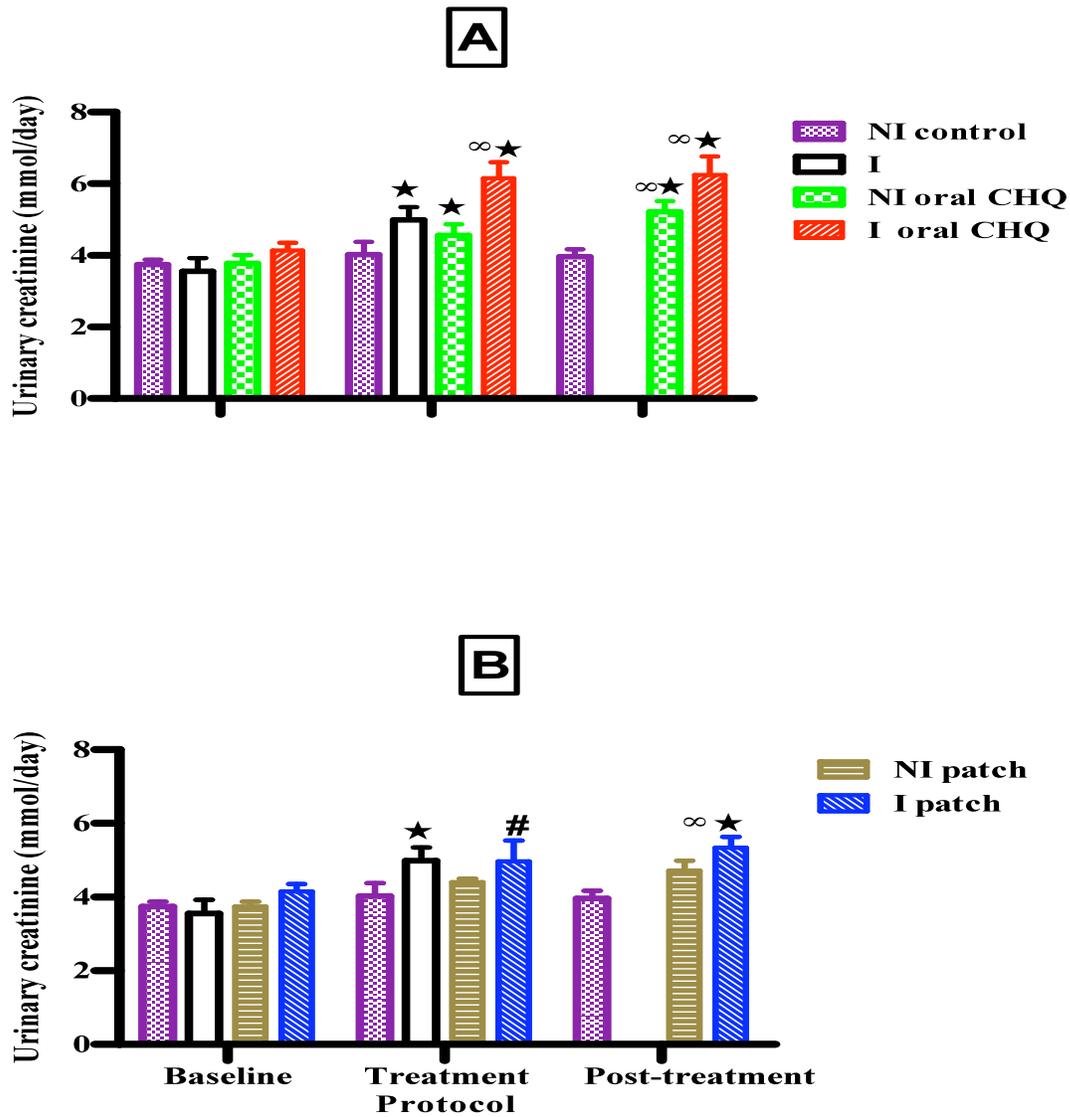
Figure 17: Comparison of the effects of twice daily treatment with oral CHQ (60mg/kg) **(A)** and with a single topically applied pectin CHQ matrix patch (53mg/kg) **(B)** on haematocrit in non infected and *P. berghei* infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group) ★p<0.05 by comparison with baseline control animals. ∞p<0.05 by comparison with non infected .control animals

5.2. MAP

5.2.1 Oral CHQ treatment

The MAP of non infected control animals remained stable throughout the 21 day experimental period (Figure 18). However, MAP of infected animals significantly decreased during the pre treatment and treatment periods by comparison to the non infected control animals. Treatment of separate groups of non infected and infected animals with oral CHQ significantly reduced MAP by comparison to non infected controls as shown in Figure 18.

MAP was not measured in the animals treated with pectin CHQ matrix patch treated animals in the current study.
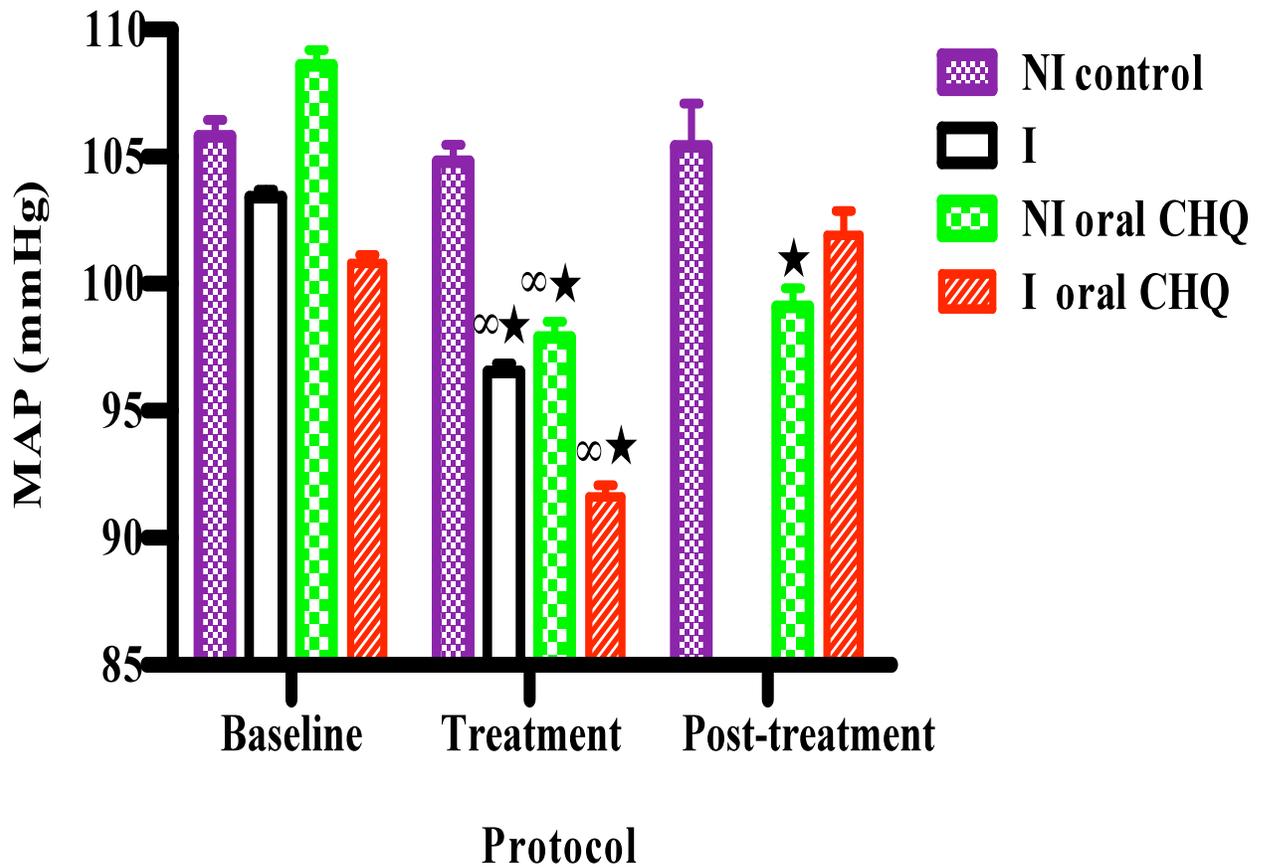
Figure 18: Comparison of the effects of twice daily treatment with oral CHQ (60mg/kg) on MAP in non infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group) ★p<0.05 by comparison with baseline control animals. ∞p<0.05 by comparison with non infected .control animals

5.3 Lipid profile

The effects of *P. berghei* infection and oral CHQ treatment on lipid profile parameters in non infected and infected rats were monitored over a 21 day experimental period divided into pre-treatment, treatment and post treatment periods. Separate groups of non fasted conscious animals (n=6) were sacrificed on days 0, 7, 8, 9, 10, 12, 14 and 21 to collect blood for the measurement of plasma lipid profile parameters.

Plasma lipid profile parameters were not measured in the animals treated with pectin CHQ matrix patch treated animals in the current study.

5.3.1 Total cholesterol

The plasma total cholesterol concentrations of the non infected controls remained stable throughout the experimental period. Infection of animals with *P. berghei* significantly increased plasma total cholesterol concentrations by comparison to non infected control animals. Treatment of non infected animals with oral CHQ did not significantly alter plasma total cholesterol concentrations by comparison to non infected animals. However, treatment of infected animals with oral CHQ significantly increased plasma total cholesterol concentrations by comparison to control animals (Figure 19)

5.3.2 LDL-cholesterol

The plasma LDL-c concentrations of the non infected controls remained stable throughout the experimental period. There were no significant differences in the plasma LDL-c concentration of non infected animals treated with CHQ. Infected animals treated with oral CHQ had significantly increased LDL-c concentrations by comparison to non infected control animals (Figure 20).

5.3.3 HDL-cholesterol

The plasma HDL-c concentrations of non infected control animals did not differ significantly throughout the experimental period. Infection of animals with *P. berghei* significantly reduced plasma HDL-c concentration in comparison to non infected control animals. Treatment of infected animals with oral CHQ significantly reduced the plasma HDL-c concentration in comparison to the non infected control animals (Figure 21).

In summary malaria infection elevated plasma Total cholesterol and LDL-c which were exacerbated by oral CHQ treatment. Malaria infection and oral CHQ also reduced haematocrit, and plasma HDL-c concentrations.
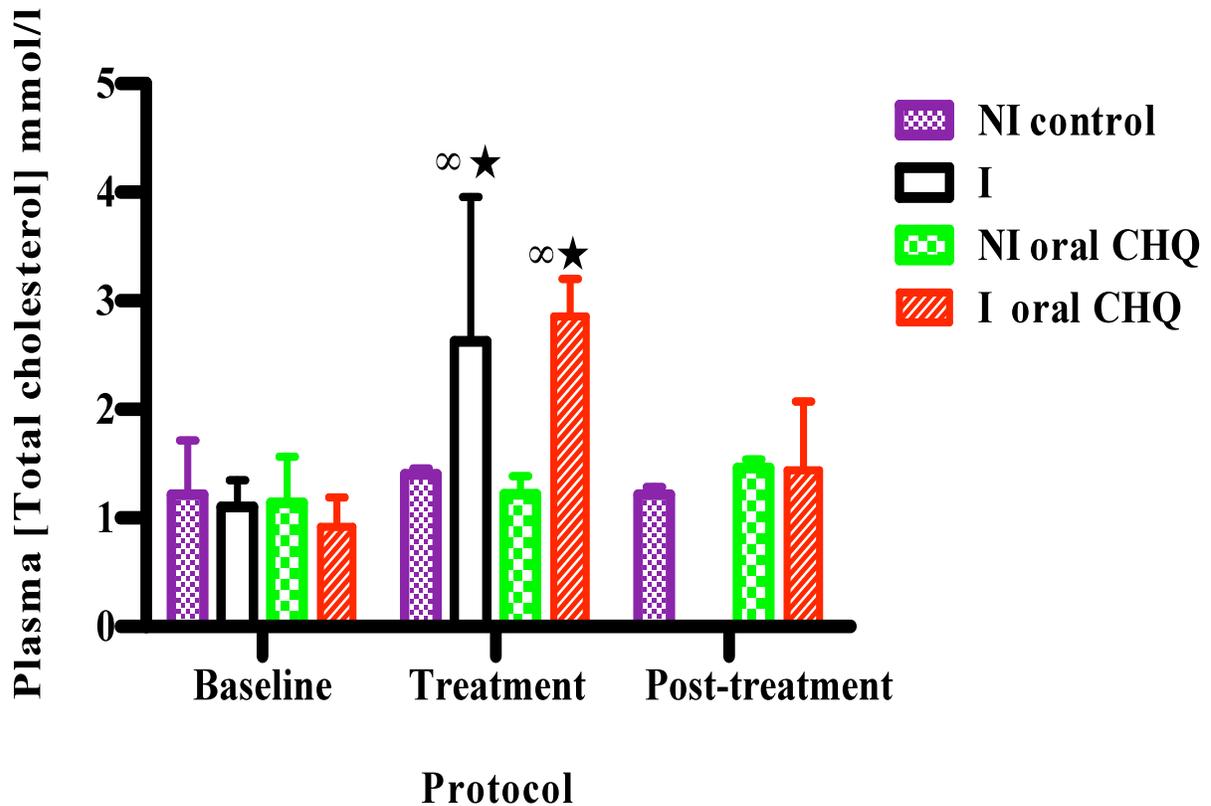
Figure 19: Comparison of the effects of twice daily treatment with oral CHQ (60mg/kg) on plasma Total cholesterol concentration in non infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group) ★p<0.05 by comparison with baseline control animals. ∞p<0.05 by comparison with non infected .control animals
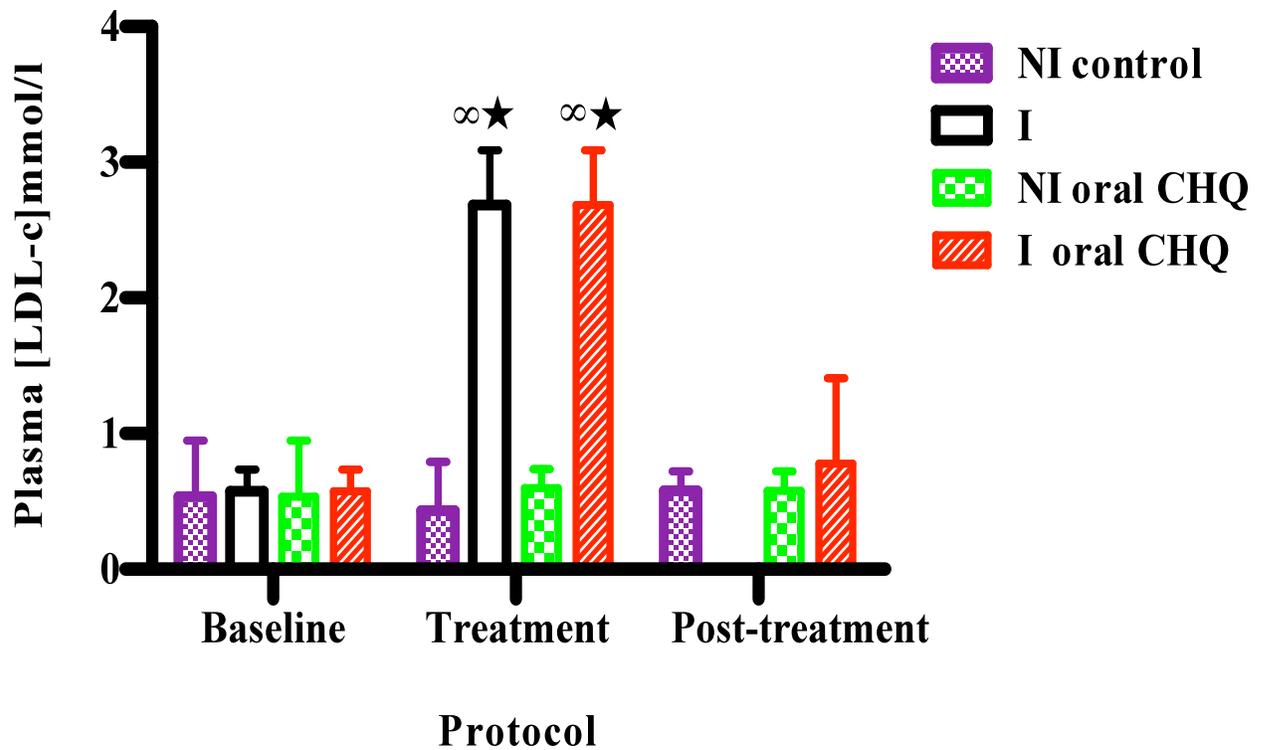
Figure 20: Comparison of the effects of twice daily treatment with oral CHQ (60mg/kg) on plasma LDL-c concentration in non infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group) ★p<0.05 by comparison with baseline control animals. ∞p<0.05 by comparison with non infected .control animals
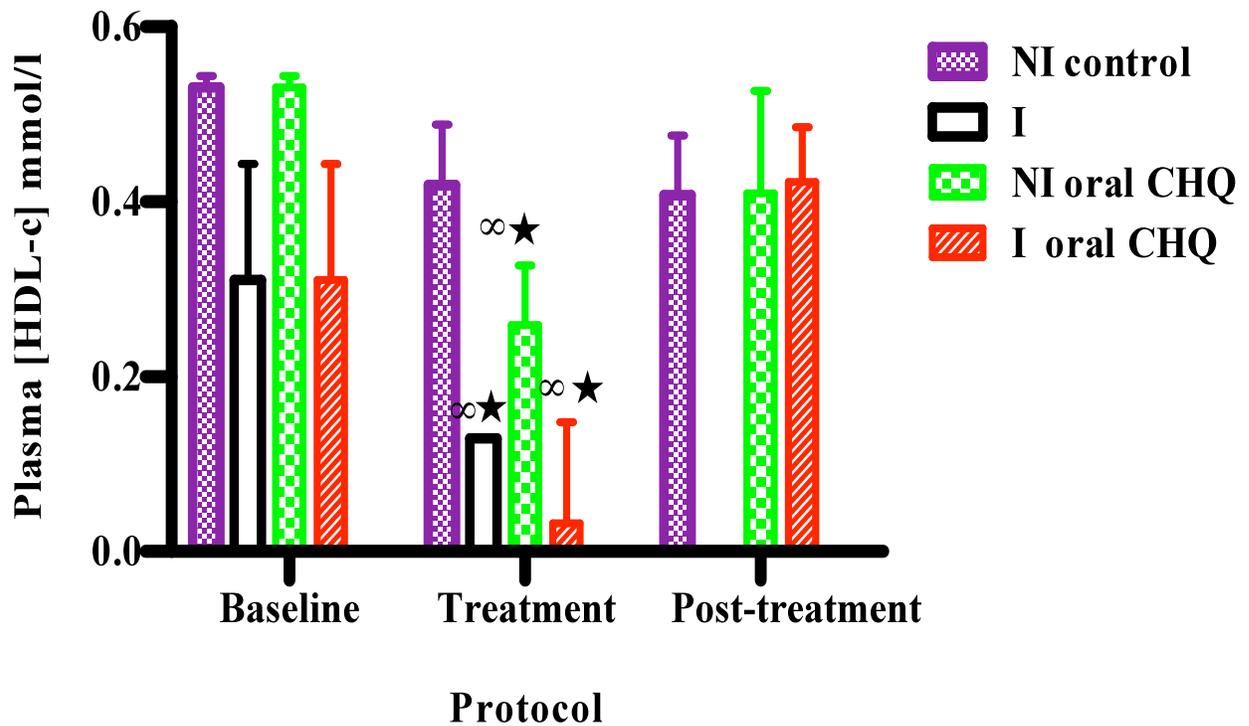
Figure 21: Comparison of the effects of twice daily treatment with oral CHQ (60mg/kg) on plasma HDL-c concentration in non infected animals (n=6 in each group). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group) ★p<0.05 by comparison with baseline control animals. ∞p<0.05 by comparison with non infected .control animals

5.4 Heart masses

The heart masses of the both the non infected and infected control groups as well as the treated groups did not differ significantly throughout the experimental period as shown in Table 4

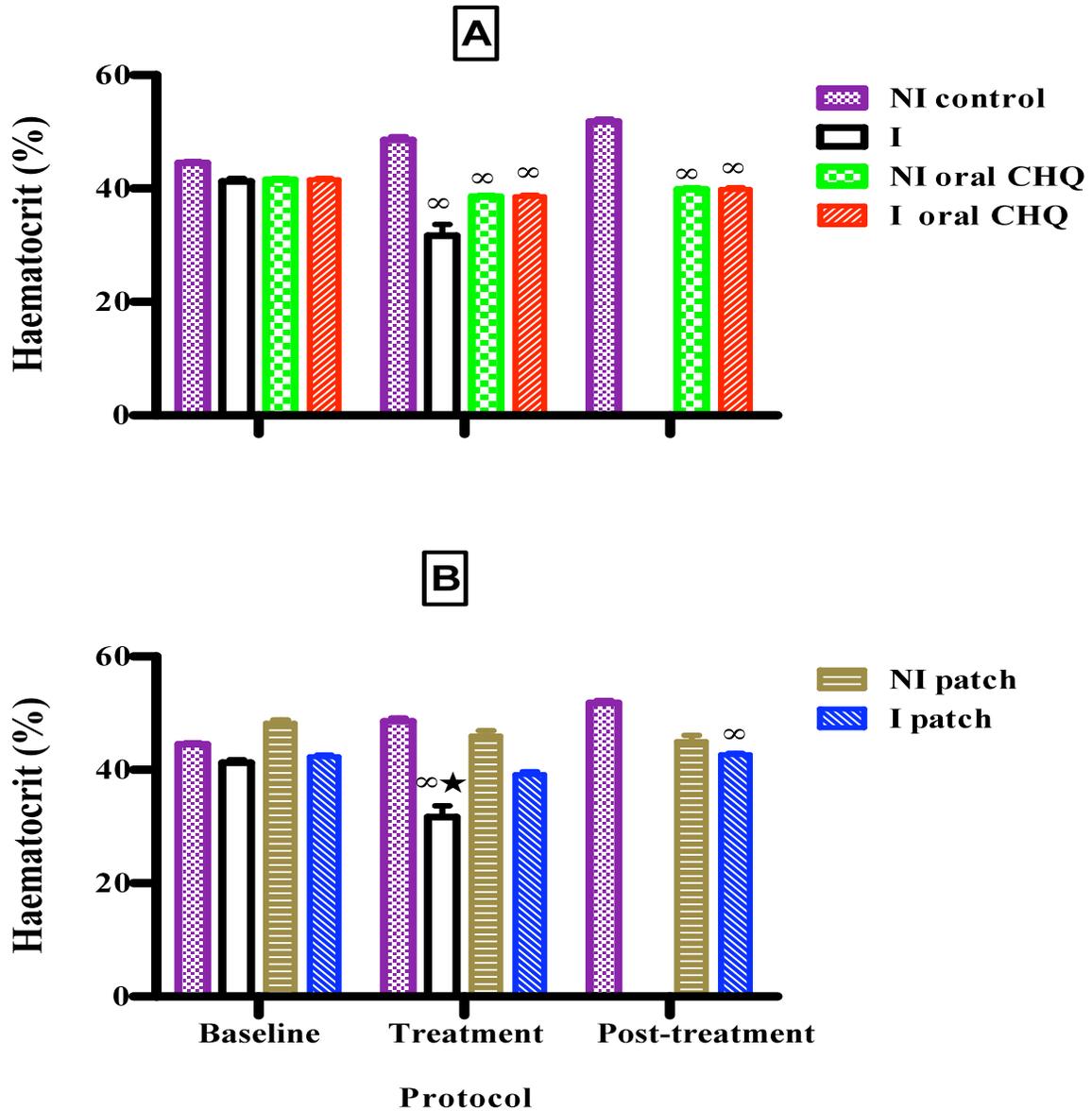**Table 4:** Comparison of the effects of twice daily treatment with oral CHQ (60mg/kg) and with a single topically applied pectin CHQ matrix patch (53mg/kg) on heart masses in non infected and *P berghei* infected animals (n=6 in each group). Values are presented as means ± SEM (n=6 in each group)

Organ (g/100g tissue)	Protocol		Pre-treatment	Treatment	Post-treatment
Heart mass	**Control**	Non infected	0.47 ± 0.02	0.43 ± 0.02	0.46 ± 0.09
		P berghei infected	0.51 ± 0.03	0.53 ± 0.09	N/A
	Oral	Non infected	0.47 ± 0.02	0.45 ± 0.43	0.43 ± 0.02
		P berghei infected	0.43 ± 0.03	0.53 ± 0.01	0.38 ± 0.01
	Patch	Non infected	0.37 ± 0.01	0.42 ± 0.01	0.40 ± 0.01
		P berghei infected	0.43 ± 0.02	0.55 ± 0.02	0.47 ± 0.04

N/A as the infected control animals were sacrificed on day 14 based on the preliminary study findings.

Chapter 6

6.0   Discussion

6.1 General

The experiments described in this thesis were designed to compare the effects of transdermal CHQ patch and oral chloroquine in the management of malaria as assessed by the ability to clear parasites of *P. berghei* infected rats. The other aim of the current study was to investigate and distinguish between the patho physiological effects of malaria and CHQ treatments on blood glucose and plasma insulin concentration, renal and cardiovascular function in male Sprague-Dawley rats. *P berghei* that was used in this study is a rodent malaria parasite that is used for experimental purposes to study human malaria as it is regarded as safe because it does not infect humans (Janse and Waters, 1995). Weanling male Sprague-Dawley rats (90-120g) were used in the current study. This is the age of rats that is highly susceptible to infection by rodent plasmodia, developing high parasitaemias that make it possible to assess the curative effects of test drugs (Dow et al., 1998; 1999; 2000). A study done by Takeuchi et al, 2011 demonstrated that the rat skin may be used as a substitute for the human skin in *in vitro* skin pemeation studies (Takeuchi et al, 2011). Animals were shaved I day prior to experimental procedures on the rat back using a shaving machine to avoid pruritis. They were placed in individual cages to avoid chances of scratching each other .The experimental animals had free access to water and standard rat chow *ad libitum* (Meadows, Pietermaritzburg, South Africa). The rats were maintained on a 12 h dark/light cycle. The biochemical measurements were done using equipment and reagents of international benchmark, as specified by the manufacturers.

The current study has demonstrated that CHQ can be delivered into systemic circulation following a once off topical application of pectin CHQ matrix patch. The results of the current study show for the first time that controlled, sustained release of therapeutic doses of CHQ were achieved, clearing all the malaria parasites after 3 days of pectin CHQ matrix patch treatment. The significance of a once off application of the CHQ patch was the ability to circumvent the

toxic effects of oral CHQ delivery in blood glucose homeostasis, renal function and cardiovascular function markers.

Thus, observations of the current study are of considerable clinical importance as they present for the first time, evidence of a novel and feasible alternative method of CHQ delivery. Topical application of the patch may ensure completion of courses of therapy as it not only masks the bitter taste of CHQ, but provides a convenient dosing schedule. Completion of courses of therapy may reduce continued development of *P. falciparum* resistance to CHQ.

6.2 Plasma CHQ pharmacokinetics

The doses used were extrapolated from clinical doses that are currently used for the prophylaxis and treatment of malaria (WHO, 2006).The peak plasma CHQ concentrations were achieved after 6 hours following CHQ administration both orally and topically. Previous studies have reported CHQ's rapid absorption following oral delivery within 4-6 hours (Walker et al., 1983, Gustafsson et al., 1983, Augustijns and Verbeke, 1993, Vries et al., 1994). Plasma CHQ concentrations decreased within 12 hours following oral CHQ delivery. This finding is in agreement with previous observations that reported the ability of CHQ to bind avidly to almost all the different epithelial cells and body tissues, attributed to the drug's large volume of distribution in the body (Gustafsson et al., 1983). Ursing et al, (2009) have also reported the ability of CHQ to accumulate in various body tissues such as the liver, lungs, spleen and kidneys (Ursing et al., 2009). The detection of CHQ in plasma after 21 days of the experimental period is in agreement with previous observations that CHQ has a long half life (McChesney et al., 1967, Adelusi and Salako, 1982).

The significance of achieving peak plasma CHQ after 6 hours following a once off topical application of pectin CHQ matrix patch is the ability to demonstrate rapid delivery of CHQ to systemic circulation from the matrix patch. The mean peak plasma CHQ concentrations following pectin CHQ matrix patch administration were sustained for a period of 3 days, suggestive of sustained, controlled release of the drug from the pectin CHQ matrix patch (Figure 1). We have previously reported the ability of the pectin CHQ matrix patch to achieve a sustained, controlled release of CHQ (Musabayane et al., 2003c).. The ability of CHQ to avidly

bind to all epithelial cells of the body has been suggested to be responsible for the adverse effects of oral CHQ on various organ systems. The shortfall, however, is that only a single dose of pectin CHQ matrix patch was used. The high plasma CHQ concentrations were sustained until day 21 suggesting that the dose used in the patch was high. There may be a need to evaluate the effect of lower doses.

6.3 Effects of CHQ treatments on:

6.3.1 Parasitaemia

Oral CHQ treatment, given twice daily, 8 hours apart for 5 continuous days and a once off topical application of CHQ, cleared the malaria parasites from systemic circulation during the treatment period. Considering that the CHQ patch was applied once, the results of the current study give evidence of sustained, controlled release of CHQ from our transdermal formulation that were able to clear the malaria parasites from systemic circulation within 3 days of treatment. Indeed, this may be a feasible alternative to conventional oral CHQ delivery of CHQ for the treatment of malaria.

6.3.2 Blood glucose and plasma insulin

P. berghei infection showed blood glucose lowering effects. Oral CHQ treatment exacerbated the blood glucose lowering effects of malaria infection. Treatment with pectin CHQ matrix patch did not alter the blood glucose concentrations of the animals throughout the 21 day experimental period. Oral CHQ treatment increased plasma insulin concentration and decreased blood glucose concentration, which were not altered by topical application of the pectin CHQ matrix patch in non infected animals. The blood glucose lowering effects of the patch acutely during the OGT responses demonstrates the sustained controlled release of CHQ from the transdermal formulation. However, the magnitude of the decline of blood glucose concentrations following patch treatment was lower, by comparison to oral treatment, suggestive of the adverse effects of oral CHQ delivery. The blood glucose lowering effects of the various doses of both oral CHQ treated and pectin CHQ matrix patch treated separate groups of animals were not significantly different from each other suggestive of a non dose dependant effect. The transdermal patch, just

like oral CHQ has demonstrated the ability to ensure rapid onset of action, when used as an adjunct to treat malaria

Indeed other investigators have reported blood glucose lowering effects of the malaria parasites. Phillips (1989) reported that the parasite heavily and completely depends on the host for all its energy requirements, which impact negatively on the host's energy store (Phillips 1989). The author went further to suggest that the overall effect may be an imbalance between the host's energy demands and supply which may precipitate hypoglycaemia in patients with glucose-insulin mismatching (Phillips 1989). Krishna et al, (1999), has previously reported that there are various mechanisms, which are currently still unclear, through which the malaria parasites may precipitate hypoglycaemia in the host. One such proposed mechanism is mediated through depletion of vital gluconeogenic substrates such as thiamine (Krishna et al., 1999). Dekker et al, (1996) concluded that the major determinant of plasma blood glucose of children with uncomplicated falciparum malaria is endogenous glucose productions as opposed to peripheral uptake as previously reported in adults (Dekker et al., 1996, Dekker et al., 1997). The author reported that gluconeogenesis is vital in the maintenance of blood glucose concentration in children with uncomplicated falciparum malaria (Dekker et al., 1996). Hypoglycaemia, which is caused by inhibition of glucose production and stimulation of glucose disposal, may therefore be more severe in children and pregnant women (Dekker et al., 1996). The hypoglycaemia in malaria patients may be attributed to low levels of gluconeogenic substrates, imbalance in the levels of hormones that maintain glucose homeostasis as well as high levels of cytokines, which is characteristic of malaria (Dekker et al., 1996).

Blood glucose production in humans can be stimulated by catecholamines, glucagon and low levels of cytokines TNF-a and IL-6. Low levels of IL-l have reportedly been shown to induce hypoglycaemia in animals (Dekker et al., 1996, Dekker et al., 1997). The direct contribution of the malaria parasite in causing hypoglycaemia has only been reported to be less than 10%, as the authors suggested that the parasite only consumes at most 10% of the total blood glucose produced by the host, indicating that other factors such as metabolic changes in the host are the most vital determinants of malaria induced hypoglycaemia (Dekker et al., 1996). In the current

study, weanling rats were used, which could also be an important factor in glucose homeostasis in a malaria model as previous studies by Dekker et al, (1996) indicated that glucose metabolism in *falciparum* malaria is differently regulated in children and adults (Dekker et al., 1996). The pathophysiology of hypoglycaemia in malaria remains unclear to date (English et al., 1998, Thien et al., 2006). Dekker et al, (1997) has also attributed malaria induced hypoglycaemia to liver damage associated with malaria . The liver damage results from the rapid proliferation and migration of malaria parasites from one hepatocyte to another during the life cycle of the malaria parasite has been shown to cause hepatocellular damage (Dekker et al., 1997). The liver is a key organ involved in the maintenance glucose homeostasis; hence any hepatocellular damage may impair glucose homeostasis. The liver plays a major role in breaking down the activated insulin-receptor complex hence hepatocellular damage may, therefore, result in slow insulin receptor recycling, thereby precipitating overt hypoglycaemia in malaria (Onyesom and Agho, 2011). The results of the current study are therefore consistent with the findings of other scholars who have reported blood glucose lowering effects of the malaria parasites.

On the other hand, besides the independent, malaria induced blood glucose lowering effects; findings of the current study indicate that CHQ also independently has blood glucose lowering effects. Indeed, this is in agreement with previous studies which have suggested that CHQ may possess hypoglycaemic properties. As already alluded to in section 1.4.2, various studies have suggested that CHQ may evoke hypoglycaemia through varied and unclear mechanisms (Knutson, Ronnett and Lane, 1985; Davis, 1997; Jarzyna, Kiersztan, Lisowa and Bryla, 2001). The suggested CHQ- induced hypoglycaemia has been ascribed to decreased insulin degradation (Knutson, Ronnett and Lane, 1985) or increased insulin secretion (Davis, 1997). Bevan et al, 1995, has reported that hypoglycaemia may be due to CHQ induced extension of the life span of CHQ-insulin activated complex (Bevan et al., 1995). CHQ, being a diprotic base, rapidly accumulates in acidic lysosomes and endosomes thereby elevating lysosomal pH. An elevation of lysosomal pH compromises the optimal activity of lysosomal hydrolases that degrade hepatic insulin, with a concomitant increase in plasma concentration of intact insulin (Knuston, Ronnet and Lane, 1985). Smith et al, 1987, has also reported that non insulin dependent diabetic patients

that were given CHQ, had increased plasma insulin concentration with a concomitant improvement in their glucose tolerance (Smith et al., 1987). CHQ has also been shown to increase insulin secretion via rapid accumulation in the pancreas, thereby having a direct effect on the beta cells of islets of Langerhans (Asamoah et al., 1990). The CHQ evoked hypoglycaemia and elevations in urinary potassium output, as well as plasma potassium observed in the current study may also be partly attributed to the fact that in the pancreatic β-cells glucose produces ATP by glycolysis. The resultant increased ATP, inhibits the K^+-ATP channel, preventing K^+ outflow which causes depolarization of the membrane (Ashcroft, 2007). Opening of voltage sensitive calcium channels follows membrane depolarization, with a consequent increase in intracellular calcium which leads to insulin secretion (Ashcroft, 2007)

CHQ induced hypoglycaemia has also been ascribed to inhibition of gluconeogenesis due to CHQ induced hepatocellular damage (Jarzyna, Kiersztan, Lisowa and Bryla, 2001). CHQ induced hepatocellular damage may lead to inhibition of key gluconeogenic enzymes; glutamate dehydrogenase and glucose-6-phosphatase (Jarzyna et al., 2001). It may also lead to decreased transport of pyruvate into hepatocyte mitochondria (Jarzyna et al., 2001) with a concomitant decrease of gluconeogenesis, which may eventually cause hypoglycaemia. The results of the current study indicate that oral CHQ delivery decrease glucose homeostasis. However, the findings of the current study did not elucidate the mechanism of both malaria and CHQ induced hypoglycaemia. The blood glucose concentrations of the animals treated with pectin CHQ matrix patch remained stable throughout the 21 day experimental period. This suggests that the sustained controlled release of CHQ did not have adverse effects on blood glucose homeostasis. The controlled and sustained release of CHQ from our transdermal formulation, therefore, averted the challenges of dose dumping associated with oral CHQ delivery. We have for the first time shown that the CHQ concentrations released from the pectin matrix patch may circumvent the adverse effects of oral CHQ in the rat.

The elevated blood glucose concentration of separate group of animals that were treated with transdermally delivered CHQ at the end of the experimental period could be attributed to increased food intake of the growing weanling rats. The other factor that may contribute to

decreased blood glucose concentration in orally treated animals is associated with the bitter taste of CHQ. The drug is unpalatable, thereby reducing the appetite of the animals. Transdermal delivery of CHQ masks the bitter taste of the drug; hence the feeding patterns of these animals did not change throughout the experimental period. We suggest that transdermal delivery of CHQ may, therefore, promote the completion of courses of therapy by malaria patients thereby reducing development of falciparum resistance to the drug.

There is currently no literature evidence showing the effects of oral CHQ and transdermal delivery on hepatic and muscle glycogen storage. This is despite the pivotal role played by the liver in blood glucose homeostasis. Glycogen determination was performed using a protocol that has been used extensively in our laboratory (Ngubane et al., 2011)

Hepatic glycogen concentration in non infected animals that were treated with oral CHQ was elevated in the current study. Indeed, we had speculated that since CHQ alters blood glucose and plasma insulin concentrations, it could be altering the hepatic and muscle glycogen stores as well. The hepatic glycogen concentrations of animals that were treated with transdermal patch remained stable throughout the 21 day study. The gastrocnemius muscle glycogen concentration of all the groups of animals remained stable throughout the experimental period. Elevations of plasma insulin and decreased blood glucose observed in this study could be partly responsible for increased hepatic glycogen concentrations. Insulin acts on insulin sensitive tissues such as the skeletal muscle, thereby promoting increased blood glucose uptake and eventually increased glycogen stores (Azpiazu et al., 2000). We, therefore, speculate that the increased plasma insulin concentrations following oral CHQ administration promoted glucose uptake in the muscle via the GLUT 4 transporters (Azpiazu et al., 2000, Ferrer et al., 2003). Possible increases in glucose uptake by the liver via GLUT 2 transporters that are insulin independent could have led to increased glycogen stores in the organ (Agius, 2008). The mechanism through which the elevation of hepatic glycogen is mediated was not established in this study. We, however, speculate based on our previous findings that this could be due to the effects of CHQ on glycogenic enzymes, glucokinase and hexokinase (Ngubane et al., 2011). Previous studies have shown that falciparum malaria causes muscle damage, which could have masked the effects of

CHQ on glycogen concentration in this organ for the malaria infected animals (Davis et al., 1999). The liver masses of the infected control animals and the oral CHQ treated infected animals were increased by comparison to non infected control animals the pectin CHQ matrix patch treated animals. This observation was consistent with previously reported CHQ induced hepatocellular damage (Cooper and Magwere, 2008). This observation indicates the toxic effects of oral CHQ that may be circumvented by slowly released, in a controlled manner of CHQ from our transdermal formulation.

6.4 Renal function

Renal function parameters were monitored every 3rd day during the pre and post treatment periods and daily during the treatment period in separate groups of non infected and infected animals treated with oral CHQ and pectin CHQ matrix patch in a 21 day experimental study.

6.4.1 Physico metabolic parameters

As already mentioned above, malaria infection and oral CHQ treatment reduced food and water intake as well as decreasing % body weight changes. The food intake, water intake and % body weight changes of non infected animals that were treated with pectin CHQ matrix patch remained stable throughout the experimental period, suggesting the ability of the transdermal formulation to circumvent the adverse effects of oral CHQ on these parameters. The reduction in food intake of oral CHQ treated animals is consistent with previous, attributing the reduced appetite to the nausea and bitter taste of CHQ (Tulpule and Krishnaswamy, 1982; Musabayane et al., 2003b). The unpalatability of CHQ possibly led to reduced body weight changes in this group of animals. When there is reduced feeding, the body may start to use other less easily accessible substrates such as fatty acids and amino acids as opposed to glucose, which may result in the observed reduction in body weight changes of the oral CHQ group (Table 2). This same phenomenon as previously mentioned may be partly responsible for the blood glucose lowering effects of oral CHQ

6.4.2 Renal fluid and electrolyte handling and plasma biochemical parameters

Infected animals had a significantly lower mean 24 hour urine output by comparison to non infected control animals. This finding agrees with reports by Das (2008) that malaria the pathogenesis of falciparum malaria leads to varying degrees of fluid and electrolyte imbalances characterized by extracellular fluid volume depletion (Boonpucknavig and Sitprija, 1979; Weller et al., 1992; English et al., 1996; Prakash et al., 1996; Prakash et al., 2002; Naqvi et al., 2003;

Das, 2008; Ogbadoyi and Tsado, 2009; Idonije et al., 2011). The mechanism leading to reduced urine output in malaria infected rats remain to be elucidated. The current study did not elucidate the mechanism.

The results of the current study have also shown elevation of mean urinary Na^+ outputs in non infected and infected animals treated with oral CHQ. These findings are consistent with previous observations that acutely administered oral CHQ stimulated AVP secretion (Musabayane et al., 2000c) We have previously shown that acute CHQ treatment causes natriuresis, without affecting urine output (Musabayane et al., 1993, Musabayane et al., 1996) CHQ reportedly inhibits AVP induced cyclic adenosine monophosphate (cAMP) production at the level of the hormone receptor complex (Musabayane et al., 2000c.) CHQ therefore, reduces the concentration of cAMP in the inner medullary collecting ducts, thereby inhibiting the antidiuretic action of the hormone (Musabayane et al., 2000c). We have previously suggested the natriuretic effects of CHQ to be mediated via AVP effects on V_1 receptors. On these receptors, AVP has pressor effects, thereby causing natriuresis without affecting urine flow rate (Musabayane et al., 2000b).

Similarly, malaria infection may induce natriuresis through a similar mechanism leading to production of hyperosmotic urine (Elsheikha and Sheashaa, 2007, Ehrich and Eke, 2007, Das, 2008). The mechanism of CHQ induced natriuresis was not elucidated in the current study, as the plasma AVP concentrations were not measured.

The current study has also shown hyperkalaemia and elevated urinary K^+ outputs in malaria infected control animals. .Elevated urinary K^+ outputs were also shown in *P berghei* infected animals treated with oral CHQ. Malaria infection is associated with haemolysis of RBC, which may release cellular K^+ into systemic circulation (Clark and Cowden, 2003; Das, 2008). The observed kaliuresis can, therefore be attributed to the increased filtered load of K^+ Topical application of pectin CHQ matrix patch did not alter the urinary Na^+ and K^+ outputs of both non infected and infected animals, suggestive of the ability of the patch to circumvent the adverse effects of oral CHQ delivery

Urinary creatinine outputs were elevated in *P berghei* infected animals treated with with oral CHQ. This observation is consistent with previous reports that *falciparum* malaria may impair renal fluid and electrolyte handling (Ekeanyanwu and Ogu, 2010). In the current study, there were no significant differences in GFR of the different experimental groups. GFR is a marker used to assess renal function (Narayanan and Appleton 1980; Smith et al 2006). Creatinine clearance (Cc) is used to estimate GFR (Narayanan and Appleton 1980; Smith et al 2006). It is speculated that other mechanisms could be influencing the observed impairments of renal fluid and electrolyte handling observed in the current study.

All the experimental groups had stable 24 hour urinary urea outputs throughout the experimental period. Blood urea concentrations may not accurately be used to assess renal function because urea production may be altered by other factors such as dehydration, food intake, and tissue catabolism (Manan et al., 2006; Ekeanyanwu and Ogu, 2010).

6.5 Cardiovascular function

6.5.1 Haematocrit

Reduction in the haematocrit of malaria infected animals by comparison to non infected group of animals is consistent with previous studies that have reported malaria induced anaemia (Menendez 2000). Indeed, anaemia is a common manifestation of malaria infection, which may indirectly influence haematocrit. Anaemia may result from pathophysiological mechanisms that promote increased destruction of infected RBCs through multiple mechanisms (Bjorkman, 2002; Clark and Cowden 2003). Decreased production of RBC associated with malaria infection may also lead to development of anaemia via varied mechanisms (Yap and Stevenson, 1994; El Hassan et al., 1997; Dondorp et al., 1999; Chang et al., 2004; Fendel et al., 2010). Oral CHQ treatment reduced haematocrit of both malaria infected and non infected animals. The observation is consistent with the previous reports suggesting quinoline drugs to act as haptens. CHQ may possibly bind to infected RBC proteins and become antigenic, eventually causing destruction of RBC, which may result in the development of anaemia (Clark and Cowden, 2003). Topical application of pectin CHQ matrix patch did not alter haematocrit of non infected animals, suggestive of the ability of the patch to circumvent the toxic effects of oral CHQ delivery

The current study did not elucidate the mechanisms through which haematocrit was reduced in the infected and oral CHQ treated animals. Measurement of plasma cytokines may give a clear mechanism of the observed malaria induced haematocrit changes.

6.5.2 Hypotensive effects of CHQ

The results of the present study have demonstrated the hypotensive effects of malaria infection which were exacerbated by oral CHQ treatment. To date, the mechanisms responsible for orthostatic hypotension in malaria remain incompletely understood (Ekue et al., 1987). Malaria induced hypotension may be mediated through development of bradycardia and redistribution of blood volume to the skin by peripheral vasodilatation (Ekue et al., 1987; Supanaranond et al., 1993; Bethel et al 1996). Indeed, *P.falciparum* infection is generally associated with

hyperpyrexia and vasodilatation of peripheral blood vessels (Supanaranond et al., 1993; Bethel et al 1996).

Hypotension associated with CHQ may be a result of arteriolar dilatation and venodilatation (Anigbogun et al., 1993). CHQ induced venodilation, mediated via the release of NO in human forearm veins has been reported ((Abiose et al, 1997). Other mechanisms that have been suggested to explain the previously reported CHQ induced hypotensive effects, were CHQ ability to cause blockade of alpha receptors and increased histamine secretion (Abiose et al, 1997). Mubagwa and Adler, 1998 have previously reported that CHQ may be invoking its actions via the muscarinic receptors (Mubagwa and Adler, 1988). The findings of this study have, therefore, demonstrated that malaria infection and oral CHQ delivery independently evoke hypotensive effects. The effects of transdermal delivery of CHQ on MAP were not measured in the current study.

6.5.3 Lipid profile parameters

Malaria infection and oral CHQ administration resulted in elevated plasma total cholesterol, LDL-c and reduction of plasma HDL-c. Reduction of the cardio protective particle, HDL-c may be a predisposing factor to premature coronary artery disease (Grundy, 1995). Reduced concentrations of plasma HDL-c may, therefore, be associated with development of atherosclerosis, which eventually lead to the development of CVD. HDL-c molecule has been shown to exert cardio protection through multiple mechanisms (Grundy, 1995; Libby, 2001; Toth, 2005; Natarajan et al., 2010). HDL has the ability to initiate reverse cholesterol transport, by removing and transporting cholesterol from cells to the liver for excretion (Grundy, 1995; Libby, 2001; Toth, 2005; Natarajan et al., 2010). .HDL is able to reduce the amount of cholesterol from the arterial walls, thereby slowing down the progression of atherosclerotic lesions (Grundy, 1995; Libby, 2001; Toth, 2005; Natarajan et al., 2010). HDL may mediate cardio protection by preventing the aggregation of LDL particles within the arterial wall or preventing the oxidation of LDL (Libby, 2001). Reduction of plasma HDL-c concentration may therefore accelerate atherogenesis. (Grundy, 1995).

Elevated plasma total cholesterol and LDL-c particles is associated with atherogenesis, which may be a predisposing factor to development of atherosclerotic plagues (Grundy, 1995; Ross, 1999). The findings of the current study suggest the need to monitor cardiovascular function in malaria patients taking CHQ. The effects of transdermal delivery of CHQ on lipid profile parameters were not measured in the current study.

6.6 Conclusions

The results of the current study show for the first time that controlled, sustained release of therapeutic doses of CHQ, were delivered into systemic circulation following a once off topical application of pectin CHQ matrix patch. The delivered CHQ doses were able to clear malaria parasites from systemic circulation.

The current study was able to distinguish between the pathophysiological effects of malaria and CHQ treatments on blood glucose and plasma insulin concentration. The study has demonstrated the independent blood glucose lowering effects of malaria infection and oral CHQ delivery which were circumvented by topical application of the pectin CHQ matrix patch. The study has also demonstrated that oral CHQ elevates hepatic glycogen storage through mechanisms that are still to be elucidated. Topical application of CHQ via pectin matrix patch did not alter hepatic and gastrocnemius muscle glycogen stores.

The current study has demonstrated the independent ability of both malaria infection and oral CHQ to impair physico metabolic parameters and renal fluid electrolyte handling. Both malaria infection and oral CHQ delivery induced natriuresis, reduced urine output and increased urinary creatinine outputs. Malaria infection was shown to cause both hyperkalaemia and kaliuresis in experimental animals.

The study has also demonstrated the hypotensive effects of malaria infection and oral CHQ delivery. Additionally, the results of the current study have also demonstrated the independent elevations of atherogenic particles, plasma total cholesterol and LDL-c as well as reduction in the cardioprotective particle, plasma HDL-c, due to malaria infection and oral CHQ delivery.

Overall, the once off application of the CHQ patch was able to circumvent the adverse effects of oral CHQ delivery in blood glucose homeostasis, renal function and cardiovascular function markers.

6.7 Shortfalls

The limitations and shortfalls of the current study included the use of a single dose was used in the short term studies to investigate and distinguish the pathophysiological effects of CHQ on glucose homeostasis, renal function and cardiovascular function. Inability to measure plasma AVP, cytokines, hs-CRP as well as hepatic and gastrocnemius muscle glycogenic enzymes hexokinase and glucokinase was a shortfall in the current study. Measurement of these parameters may help to elucidate the mechanisms for some of the findings of the current study. Another shortfall of the study was lack of histochemical analysis of the harvested organs yet; such analysis may give more information, pertaining to the possible adverse effects of oral CHQ treatment on the organ systems

6.8 Future work and recommendations

The future direction from the current study would be the use of various doses of CHQ in the pectin CHQ matrix patch, which may be useful in optimizing the transdermal formulation. Measurement of AVP may help in elucidating the exact mechanisms for the observed reduction in urine output and natriuresis in malaria infected animals treated with CHQ. Future work could also include measurement of plasma cytokines, which may give clearer mechanisms to explain the observed differences in haematocrit. The mechanisms responsible for the elevated hepatic glycogen concentrations were not established in the present study. We speculated that measurement of glycogenic enzymes hexokinase and glucokinase could give a possible explanation. Histochemical analysis of the harvested organs may also give more information, pertaining to the possible adverse effects of oral CHQ treatment on the organ systems

CHAPTER 7-REFERENCES

ABIOSE, A. K., GROSSMANN, M., TANGPHAO, O., HOFFMAN, B. B. & BLASCHKE, T. F. 1997. Chloroquine induced vasodilation in human hand veins. *Clinical Pharmacology and Therapeutics,* 61**,** 677-683.

ADELUSI, S. A., DAWODU, A. H. & SALAKO, I. A. 1982. Kinetics of the uptake and elimination of chloroquine in children with malaria. *British . Journal of . clinical. Pharmacology. ,* 14**,** 483-487.

ADELUSI, S. A. & SALAKO, L. A. 1982. Tissue and blood concentrations of chloroquine following chronic administration in the rat. *Journal of Pharmacy and Pharmacology,* 34**,** 733-5.

AGIUS, L. 2008. Glucokinase and molecular aspects of liver glycogen metabolism. *Biochemical Journal,* 414**,** 1-18.

AHMED, M. H., ASHTON, N. & BALMENT, R. J. 2003a. The effect of chloroquine on renal function and vasopressin secretion: a nitric oxide-dependent effect. *Journal of Pharmacology and Experimental Therapeutics,* 304**,** 156-61.

AHMED, M. H., BALMENT, R. J. & ASHTON, N. 2003b. Renal action of acute chloroquine and paracetamol administration in the anesthetized, fluid-balanced rat. *Journal of Pharmacology and Experimental Therapeutics,* 306**,** 478-83.

ALSHEIKH-ALI, A. A., KUVIN, J. T. & KARAS, R. H. 2005. High-density lipoprotein cholesterol in the cardiovascular equation: Does the "good" still count? *Atherosclerosis* 180**,** 217-223.

ANIGBOGUN, C. N., ADIGUN, S. A., I., I. & ADEGUNLOYE, B. J. 1993. Chloroquine reduces blood pressure and forearm vascular resistance and increases forearm flow in healthy young adults. *Clinical Physiology,* 13**,** 209-216.

ARUN KUMAR, C. & DAS, U. N. 1999. Lipid peroxides, nitric oxide and essential fatty acids in patients with Plasmodium falciparum malaria. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 61, 255-8.

ASAMOAH, K. A., ROBB, D. A. & FURMAN, B. L. 1990. Chronic chloroquine treatment enhances insulin release in rats. *Diabetes Research and Clinical Practice,* 9, 273-8.

ASHCROFT, F. M. 2007. ATP-sensitive K+ channels and disease: from molecule to malady. *American Journal of Physiology Endocrinology and Metabolism,* 293**,** E880-E889

AUGUSTIJNS, P. & VERBEKE, N. 1993. Stereoselective pharmacokinetic properties of chloroquine and de-ethyl-chloroquine in humans. *Clinical Pharmacokinetics,* 24**,** 259-69.

AUNGST, B. J. 1989. Structure/effect studies of fatty acid isomers as skin penetration enhancers and skin irritants. *Pharmaceutical Research,* 6**,** 244-7.

AZPIAZU, I., MANCHESTER, J., SKURAT, A. V., ROACH, P. J. & LAWRENCE, J. C., JR. 2000. Control of glycogen synthesis is shared between glucose transport and glycogen synthase in skeletal muscle fibers. *American Journal of Physiology Endocrinology and Metabolism,* 278**,** E234-43.

BAIRD, J. K. 2000. Resurgent malaria at the millennium: control strategies in crisis. *Drugs,* 59**,** 719-43.

BARENNES, H., PUSSARD, E., SANI, A. M., CLAVIER, F., KAHIATANI, F., GRANIC, G., HENZEL, D., RAVINET, L. & VERDIER, F. 1996. Efficacy and pharmacokinetics of a new intrarectal quinine formulation in children with Plasmodium falciparum malaria. *British . Journal of . clinical. Pharmacology.,* 41 (5)**,** 389–395.

BARRETT, C. W. 1969. Skin penetration. *Journal Society of cosmetic chemists,* 20**,** 487-499.

BARRY, B. W. 1991. Modern methods of promoting drug absorption through the skin. *Molecular Aspects of Medicine,* 12**,** 195-241.

BARRY, B. W. 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. *European Journal of Pharmaceutical sciences,* 14**,** 101-114.

BARSOUM, R. S. 2000. Malarial acute renal failure. *Journal of the American Society of Nephrology,* 11**,** 2147-54.

BATEMAN, D. N., BLAIN, P. G., WOODHOUSE, K. W., RAWLINS, M. D., DYSONS, H., HEYWORTH, R., PRESCOTTJ, L. F. & PROUDFOOT, A. T. 1985. Pharmacokinetics and Clinical Toxicity of Quinine Overdosage: Lack of Efficacy of Techniques Intended to Enhance Elimination. *Quarterly Journal of Medicine, New Series 54,* 214**,** 125-131.

BENAVIDES-HARO, D. E. & SÁNCHEZ-CHAPULA, J. A. 2000. Chloroquine blocks the background potassium current in guinea pig atrial myocytes. *Naunyn-Schmiedeberg's Archives of Pharmacology,* 361**,** 311–318.

BENSON, H. A. 2005. Transdermal drug delivery: penetration enhancement techniques. *Current Drug Delivery,* 2**,** 23-33.

BETHEL, D. B., PHUONG, P. T., PHUON, C. X. T., NOSTEN, F., WALLER, D., DAVIS, T. M. E., DAY, N. P. J., CRAWLEY, J., BREWSTE, D., PUKRITTAYAKAMEE, S. & WHITE, N. J. 1996. Electrocardiographic monitoring in severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 90**,** 266-269.

BEVAN, A. P., KROOK, A., TIKERPAE, J., SEABRIGHT, P. J., SIDDLE, K. & SMITH, G. D. 1997. Chloroquine Extends the Lifetime of the Activated Insulin Receptor Complex in Endosomes. *The Journal of Biological Chemistry,* 272**,** 26833-26840.

BEVAN, P. A., CHRISTENSEN, J. R., TIKERPAE, J. & SMITH, G. D. 1995. Chloroquine augments the binding of insulin to its receptor. *Biochemistry Journal,* 311**,** 787-795.

BEYNEN, A. C. 1986. Could chloroquine be of value in the treatment of hypercholesterolemia? *Artery,* 13**,** 340-51.

BIAGINI, G. A., O'NEILL, P. M., NZILA, A., WARD, S. A. & BRAY, P. G. 2003. Antimalarial chemotherapy: young guns or back to the future? *TRENDS in Parasitology* 19**,** 479-487.

BJORKMAN, A. 2002. Malaria associated anaemia, drug resistance and antimalarial combination therapy. *International Journal for Parasitology* 32**,** 1637–1643.

BLOLAND, P. B. 2003. A contrarian view of malaria therapy policy in Africa. . *The American Journal of Tropical Medicine and Hygiene,* 68 (2)**,** 125–126.

BLOLAND, P. B., LACKRITZ, E. M., KAZEMBE, P. N., WERE, J. B., STEKETEE, R. & CAMPBELL, C. C. 1993. Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *Jornal of Infectious Diseases,* 167**,** 932-7.

BOND, J. R. & BARRY, B. W. 1986. Limitations of hairless mouse skin as a model for in vitro permeation studies through human skin: hydration damage. *Journal of Investigative Dermatology. ,* 90**,** 486–489.

BOND, J. R. & BARRY, B. W. 1988. Limitations of hairless mouse skin as a model for in vitro permeation studies through human skin: hydration damage. *Journal of Investigative Dermatology,* 90**,** 486-9.

BORBA, E. F. & BONFA, E. 2001. Longterm beneficial effect of chloroquine diphosphate on lipoprotein profile in lupus patients with and without steroid therapy. *Journal of Rheumatology,* 28**,** 780-5.

BOUWSTRA, J. A. & HONEYWELL-NGUYEN, P. L. 2002. Skin structure and mode of action of vesicles. *Advanced Drug Delivery Reviews,* 54 Suppl 1**,** S41-55.

BOONPUCKNAVIG, V. & SITPRIJA, V. 1979. Renal disease in acute Plasmodium falciparum infection in man. *Kidney International,* 16**,** 44-52.

BRAY, P. G., JANNEH, O., RAYNES, K. J., MUNGTHIN, M., GINSBURG, H. & WARD, S. A. 1999. Cellular Uptake of Chloroquine Is Dependent on Binding to Ferriprotoporphyrin IX and Is Independent of NHE Activity in Plasmodium falciparum. *The Journal of Cell Biology,* 145**,** 363-376.

BRAY, P. G., MUNGTHIN, R., RIDLEY, R. G. & WARD, S. A. 1998. Access to Hematin: The Basis of Chloroquine Resistance. *Molecular Pharmacology,* 54**,** 170-179.

BURKOTH, T. L., BELLHOUSE, B. J., HEWSON, G., LONGRIDGE, D. J., MUDDLE, A. G. & SARPHIE, D. F. 1999. Transdermal and transmucosal powdered drug delivery. *Critical Reviews™ in Therapeutic Drug Carrier Systems,* 16**,** 331-84.

BUSTOS, M. D., GAY, F., DIQUET, B., THOMARE, P. & WAROT, D. 1994. The pharmacokinetics and electrocardiographic eff ects of chloroquine in healthy subjects. *Annals of Tropical Medicine and Parasitology,* 45**,** 83–86.

BUTCHER, G. A. 1997. Antimalarial Drugs and The Mosquito Transmission of *Plasmodium. International Journal for Parasitology,* 27**,** 975-987.

BYL, N. N. 1995. The use of ultrasound as an enhancer for transcutaneous drug delivery: phonophoresis. *Physical Therapy,* 75**,** 539-53.

BZIK, D., LI, W., HORII, T. & INSELBURG, J. 1987. Molecular cloning and sequence analysis of the Plasmodium falciparum dihydrofolate reductase-thymidylate synthase gene. *Proceedings of the National Academy of Sciences of the United States of America,* 84**,** 8360–8364.

CAL, K., JANICKI, S. & SZNITOWSKA, M. 2001. In vitro studies on penetration of terpenes from matrix-type transdermal systems through human skin. *International Journal of Pharmaceutics,* 224**,** 81-8.

CEVC, G. 1996. Transfersomes, liposomes and other lipid suspensions on the skin: permeation enhancement, vesicle penetration, and transdermal drug delivery. *Critical Reviews in Therapeutic Drug Carrier Systems,* 13**,** 257-388.

CEVC, G. 2003. Transdermal drug delivery of insulin with ultradeformable carriers. *Clinical Pharmacokinetics,* 42, 461-74.

CEVC, G., BLUMEB, G., SCHTITZLEIN, A., GEBAUER, D. & PAUL, A. 1996. The skin: a pathway for systemic treatment with patches and lipid-based agent carriers. *Advanced Drug Delivery Reviews,* 18**,** 349-378.

CHANG, K., TAM, M. & STEVENSON, M. M. 2004. Inappropriately low reticulocytosis in severe malarial anemia correlates with suppression in the development of late erythroid precursors. *Blood,* 103**,** 3727-3735.

CLARK, I. A. & COWDEN, W. B. 2003. The pathophysiology of falciparum malaria. *Pharmacology & Therapeutics,* 99, 221-60.

CLARK, I. A. & SCHOFIELD, L. 2000. Pathogenesis of Malaria. *Parasitology Today,* 16**,** 451-454.

COOPER, R. G. & MAGWERE, T. 2008. Chloroquine: Novel uses & manifestations *Indian Journal of medical research,* 127**,** 305-316.

CROSS, S. E. & ROBERTS, M. S. 2004. Physical enhancement of transdermal drug application: is delivery technology keeping up with pharmaceutical development? *Current Drug Delivery,* 1**,** 81-92.

CUMMINGS, J. H., SOUTHGATE, D. A., BRANCH, W. J., WIGGINS, H. S., HOUSTON, H., JENKINS, D. J., JIVRAJ, T. & HILL, M. J. 1979. The digestion of pectin in the human gut and its effect on calcium absorption and large bowel function. *British Journal of Nutrition,* 41**,** 477-85.

DAS, B. S. 2008. Renal failure in malaria. *Journal of Vector Borne Diseases,* 45**,** 83-97.

DAVIS, T. M. 1997. Antimalarial drugs and glucose metabolism. *British Journal of Clinical Pharmacology,* 44**,** 1-7.

DAVIS, T. M., PONGPONRATAN, E., SUPANARANOND, W., PUKRITTAYAKAMEE, S., HELLIWELL, T., HOLLOWAY, P. & WHITE, N. J. 1999. Skeletal muscle involvement in falciparum malaria: biochemical and ultrastructural study. *Clinical Infectious Diseases,* 29**,** 831-5.

DAY, N. & DONDORP, A. M. 2007. The Management of Patients with Severe Malaria. *American Society of Tropical Medicine and Hygiene,* 77**,** 29-35.

DAYAN, N. & TOUITOU, E. 2000. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. *Biomaterials,* 21**,** 1879-85.

DEEN, J. L., SEIDLEIN, L. & DONDORP, A. 2008. Therapy of uncomplicated malaria in children: a review of treatment principles, essential drugs and current recommendations. *Tropical Medicine and International Health,* 13**,** 1111–1130.

DEKKER, E., HELLERSTEIN, M. K., ROMIJN, J. A., NEESE, R. A., PESHU, N., ENDERT, E., MARSH, K. & SAUERWEIN, H. P. 1997. Glucose homeostasis in children with falciparum malaria: precursor supply limits gluconeogenesis and glucose production. *Journal of Clinical Endocrinology and Metabolism,* 82**,** 2514-2521.

DEKKER, E., ROMIJN, J. A., WARUIRU, C., ACKERMANS, M. T., WEVERLING, G. J., SAUERWEIN, R. W., ENDERT, E., PESHU, N., MARSH, K. & SAUERWEIN, H. P. 1996. The relationship between glucose production and plasma glucose concentration in children with falciparum malaria. *Royal Society of Tropical Medicine and Hygiene,* 90**,** 654-7.

DONDORP, A. M., ANGUS, B. J., CHOTIVANICH, K., SILAMUT, K., RUANGVEERAYUTH, R., HARDEMAN, M. R., KAGER, P. A., VREEKEN, J. & WHITE, N. J. 1999 Red Blood Cell Deformability as a Predictor of Anemia in Severe Falciparum Malaria. *American. Journal of. Tropical. Medicine.and Hygiene,* 60**,** 733–737.

DONDORP, A. M., KAGER, P. A., VREEKEN, J. & WHITE, N. J. 2000. Abnormal Blood Flow and Red Blood Cell Deformability in Severe Malaria. *Parasitology Today,* 16**,** 228-232.

DONDORP, A. M., PONGPONRATN, E. & WHITE, N. J. 2004. Reduced microcirculatory flow in severe falciparum malaria: pathophysiology and electron-microscopic pathology *Acta Tropica* 89**,** 309-317.

DORMER, P., DIETRICH, M., KERN, P. & HORSTMANN, R. D. 1983. Ineffective Erythropoiesis in Acute Human P. Falciparum Malaria *Blut,* 46**,** 279-288.

DOW, G. S., O'HARA, A. J., NEWTON, S. C., REYNOLDSON, J. A. & THOMPSON, R. C. 2000. Plasmodium berghei: the antimalarial activity of albendazole in rats is mediated via effects on the hematopoietic system. *Experimental Parasitology,* 94**,** 259-63.

DOW, G. S., REYNOLDSON, J. A. & ANDREW THOMPSON, R. C. 1999. Plasmodium berghei: a new rat model for assessment of blood schizonticidal activity. *Experimental Parasitology,* 93**,** 92-4.

DOW, G. S., REYNOLDSON, J. A. & THOMPSON, R. C. 1998. Plasmodium berghei: in vivo efficacy of albendazole in different rodent models. *Experimental Parasitology,* 88**,** 154-6.

EGAN, T. J., ROSSA, D. C. & ADAMS, P. A. 1994. Anti-malarial drugs inhibit spontaneous formation of β-haematin (malaria pigment). *FEBS Letters* 352**,** 54-57.

EHRICH, J. H. H. & EKE, F. U. 2007. Malaria-induced renal damage: facts and myths. *Pediatric Nephrology,* 22**,** 626-637.

EKEANYANWU, R. C. & OGU, G. I. 2010. Assessment of renal function of Nigerian children infected with Plasmodium falciparum. *International Journal of Medicine and Medical Sciences* 2**,** 251-255.

EKUE, J. M. K., PHIRI, D. E. D. & MUKUNYANDELA, M. 1988. Severe orthostatic hypotension during treatment of falciparum malaria. *British Medical Journal,* 296**,** 396.

EL-KATTAN, A. F., ASBILL, C. S. & MICHNIAK, B. B. 2000. The effect of terpene enhancer lipophilicity on the percutaneous permeation of hydrocortisone formulated in HPMC gel systems. *International Journal of Pharmaceutics,* 198**,** 179-89.

EL HASSAN, A. M. A., SAEED, A. M., FANDREY, J. & JELKMANN, W. 1997. Decreased erythropoietin response in Plasmodium falciparum malaria-associated anaemia. *European Journal of Haernatology,* 59**,** 299-304.

EL MAGHRABY, G. M. 2008. Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion: effects of cosurfactants. *International Journal of Pharmaceutics,* 355**,** 285-92.

ELSHEIKHA, H. M. & SHEASHAA, H. A. 2007. Epidemiology, pathophysiology, management and outcome of renal dysfunction associated with plasmodia infection. *Parasitology Research,* 101**,** 1183-90.

ENGLISH, M., WALE, S., BINNS, G., MWANGI, I., SAUERWEIN, H. & MARSH, K. 1998. Hypoglycaemia on and after admission in Kenyan children with severe malaria. *Quarterly Journal of Medicine,* 91**,** 191-7.

ENGLISH, M. C., WARUIRU, C., LIGHTOWLER, C., MURPHY, S. A., KIRIGHA, G. & MARSH, K. 1996. Hyponatraemia and dehydration in severe malaria. *Archives of Disease in Childhood,* 74**,** 201-5.

ESTEVE, E., RICART, W. & FERNA´NDEZ-REAL, J. M. 2005. Dyslipidemia and inflammation: an evolutionary conserved mechanism. *Clinical Nutrition* 24**,** 16-31.

FENDEL, R., BRANDTS, C., RUDAT, A., KREIDENWEISS, A., STEUR, C., APPELMANN, I., RUEHE, B., SCHRO¨ DER, P., BERDEL, W. E., KREMSNER, P. G. & MORDMULLER, B. 2010. Hemolysis Is Associated with Low Reticulocyte Production Index and Predicts Blood Transfusion in Severe Malarial Anemia. *PLoS ONE,* 5**,** 1-8.

FERRER, J. C., FAVRE, C., GOMIS, R. R., FERNANDEZ-NOVELL, J. M., GARCIA-ROCHA, M., DE LA IGLESIA, N., CID, E. & GUINOVART, J. J. 2003. Control of glycogen deposition. *FEBS Lett,* 546**,** 127-32.

FITCH, C. D. & RUSSELL, N. V. 2006. Accelerated Denaturation of Hemoglobin and the Antimalarial Action of Chloroquine. *Antimicrobial Agents and Chemotherapy,* 50**,** 2415-2419.

FIVELMAN, Q. L., WALDEN, J. C., SMITH, P. J., FOLB, P. I. & BARNES, K. I. 1999. The effect of artesunate combined with standard antimalarials against chloroquine-sensitive and chloroquine-resistant strains of Plasmodium falciparum in vitro. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 93**,** 429-432.

FOLDVARI, M. 2000. Non-invasive administration of drugs through the skin: challenges in delivery system design. *Pharmaceutical Science & Technology Today,* 3, 417-425.

FOLEY, M. & TILLEY, L. 1998. Quinoline Antimalarials: Mechanisms of Action and Resistance and Prospects for New Agents. *Pharmacology & Therapeutics. ,* 79**,** 55-87.

FRANCIS, G. A. 2010. The complexity of HDL. *Biochimica et Biophysica Acta* 1801**,** 1296-1293.

FRANCIS, S. E., SULLIVAN, D. J., JR. & GOLDBERG, D. E. 1997. Hemoglobin metabolism in the malaria parasite Plasmodium falciparum. *Annual Review of Microbiology,* 51**,** 97-123.

FRIEDEWALD, W. T., LEVY, R. I. & FREDRICKSON, D. S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative centrifuge. *Clinical chemistry,* 18**,** 499-500.

FUNKE, A. P., SCHILLER, R., MOTZKUS, H. W., GUNTHER, C., MULLER, R. H. & LIPP, R. 2002. Transdermal delivery of highly lipophilic drugs: in vitro fluxes of antiestrogens, permeation enhancers, and solvents from liquid formulations. *Pharmaceutical Research,* 19**,** 661-8.

GONDWE, M., KAMADYAAPA, D. R., TUFTS, M., CHUTURGOON, A. A. & MUSABAYANE, C. T. 2008. Sclerocarya birrea [(A. Rich.) Hochst.] [Anacardiaceae] stem-bark ethanolic extract (SBE) modulates blood glucose, glomerular filtration rate (GFR) and mean arterial blood pressure (MAP) of STZ-induced diabetic rats. *Phytomedicine,* 15**,** 699-709.

GAMBA, G. 2010. The nanopeptide hormone vasopressin is a new player in the modulation of renal Na + − Cl − cotransporter activity. *Kidney International* 78**,** 127 − 129.

GAU, G. T. & WRIGHT, R. S. 2006. Pathophysiology, Diagnosis, and Management of Dyslipidemia. *Current Problems in Cardiology,* 31**,** 445-486.

GHOSH, K. & GHOSH, K. 2007. Pathogenesis of anemia in malaria: a concise review. *Parasitology Research,* 101**,** 1463-1469.

GOLDBERG, D. E., SLATER, A. F., BEAVIS, R., CHAIT, B., CERAMI, A. & HENDERSON, G. B. 1991. Hemoglobin degradation in the human malaria pathogen Plasmodium falciparum: a catabolic pathway initiated by a specific aspartic protease. *The Journal of Experimental Medicine,* 173**,** 961-9.

GOODMAN, M. & BARRY, B. W. 1989. Lipid– protein-partitioning theory (LPP) theory of skin enhancer activity: finite dose technique,. *International Journal of Pharmaceutics,* 57**,** 29– 40.

GORDON, S. M., HOFMANN, S., ASKEW, D. S. & DAVIDSON, W. S. 2011. High density lipoprotein: it's not just about lipid transport anymore. *Trends in Endocrinology and Metabolism* 22**,** 9-15.

GREENWOOD, B. M., FIDOCK, D. A., KYLE, D. E., KAPPE, S. H. I., ALONSO, P. L., COLLINS, F. H. & DUFFY, P. E. 2008. Malaria: progress, perils, and prospects for eradication. *The Journal of Clinical Investigation,* 118**,** 1266-1276.

GRIDER, J., FALCONE, J., KILPATRICK, E., OTT, C. & JACKSON, B. 1996. Effect of luminal vasopressin on NaCl transport in the medullary thick ascending limb of the rat. *European Journal of Pharmacology,* 313**,** 115-8.

GRUNDY, S. M. 1995. Atherogenic Dyslipidemia:Lipoprotein Abnormalities and Implications for Therapy. *The American Journal of Cardiology,* 75**,** 45B-52B.

GUMEDE, B., FOLBB, P. & RYFFELA, B. 2003. Oral artesunate prevents Plasmodium berghei Anka infection in mice. *Parasitology International,* 52**,** 53–59.

GUSTAFSSON, L. L., WALKER, O., ALVAN, G., BEERMANN, B., ESTEVEZ, F., GLEISNER, L., LINDSTROM, B. & SJOQVIST, F. 1983. Disposition of chloroquine in man after single intravenous and oral doses. *British Journal of Clinical Pharmacology,* 15, 471-9.

GUY, R. H. 1998. Iontophoresis--recent developments. *Journal of Pharmacy and Pharmacology ,* 50**,** 371-4.

HARRISON, J. E., WATKINSON, A. C., GREEN, D. M., HADGRAFT, J. & BRAIN, K. 1996. The relative effect of Azone and Transcutol on permeant diffusivity and solubility in human stratum corneum. *Pharmaceutical Research,* 13**,** 542-6.

HODIS, H. N., QUISMORIO, F. P., JR., WICKHAM, E. & BLANKENHORN, D. H. 1993. The lipid, lipoprotein, and apolipoprotein effects of hydroxychloroquine in patients with systemic lupus erythematosus. *Journal of Rheumatology,* 20**,** 661-5.

HOFFMAN, R. M. 2000. The hair follicle as a gene therapy target. *Nat Biotechnol,* 18**,** 20-1.

HOLVOET, P. & COLLEN, D. 1998. Oxidation of low density lipoproteins in the pathogenesis of atherosclerosis. *Atherosclerosis* 137**,** S33-S38.

IDONIJE, B. O., NWOKE, E. O., FESTUS, O. & OLUBA, O. M. 2011. Plasma Concentrations of Kidney Function Indicators in Malaria Patients in Ekpoma, South South Nigeria. *International Journal of Tropical medicine and hygiene,* 6**,** 4-7.

JAIN, A. K., THOMAS, N. S. & PANCHAGNULA, R. 2002. Transdermal drug delivery of imipramine hydrochloride. I. Effect of terpenes. *Journal of Controlled Release,* 79**,** 93-101.

JANSE, C. J. & WATERS, A. P. 1995. Plasmodium berghei: The Application of Cultivation and Purification Techniques to Molecular Studies of Malaria Parasites. *Parasitology Today,* 11**,** 138-143.

JARZYNA, P., KIERSZTAN, A., LISOWA, O. & BRYLA, J. 2001. The inhibition of gluconeogenesis by chloroquine contributes to its hypoglycaemic action. *European Journal of Pharmacology,* 428**,** 381-388.

JASON, J., ARCHIBALD, L., NWANYANWU, O. C., BELL, M., BUCHANAN, I., LARNED, J., KAZEMBE, P. N., DOBBIE, H., PAREKH, B., BYRD, M. G., EICK, A., HAN, A. & JARVIS, W. R. 2001. Cytokines and Malaria Parasitemia. *Clinical Immunology,* 100**,** 208-218.

JEANS, C. W. & HEARD, C. M. 1999. A therapeutic dose of primaquine can be delivered across excised human skin from simple transdermal patches. *International Journal of Pharmaceutics,* 189**,** 1-6.

JIMMY, E. O., ETIM, O. E. & USOH, F. I. 2007. Hypo and hyperglycaemia; indicators for comparative physiologic evaluation of chloroquine, fansidar, malareich and maloxine. *Acta Pharmaceutica Sceincia,* 49**,** 65-70.

JIN, W., MARCHADIER, D. & RADER, D. J. 2002. Lipases and HDL metabolism. *TRENDS in Endocrinology & Metabolism* 13**,** 174-178.

KALIA, Y. N., ALBERTI, I., SEKKAT, N., CURDY, C., NAIK, A. & GUY, R. H. 2000. Normalization of stratum corneum barrier function and transepidermal water loss in vivo. *Pharmaceutical Research,* 17**,** 1148-50.

KANIKKANNAN, N., KANDIMALLA, K., LAMBA, S. S. & SINGH, M. 2000. Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. *Current Medicinal Chemistry,* 7**,** 593-608.

KANIKKANNAN, N. & SINGH, M. 2002. Skin permeation enhancement effect and skin irritation of saturated fatty alcohols. *International Journal of Pharmaceutics,* 248**,** 219-28.

KARBWANG, J. & HARINASUTA, T. 1992. Overview: clinical pharmacology of antimalarials. *Southeast Asian Journal of Tropical Medicine and Public Health,* 23 Suppl 4**,** 95-109.

KAUL, D. K., NAGEL, R. L., LLENA, J. F. & SHEAR, D. L. 1994. Cerebral Malaria in Mice: Demonstration of Cytoadherence of Infected Red Blood Cells and Microrheological Correlates. *The American Society of Tropical Medicine and Hygiene,* 50**,** 512-521.

KAUL, D. K., ROTH, E. F., NAGEL, R. L., HOWARD, R. J. & HANDUNNETTI, S. M. 1991. Rosetting of Plasmodium falciparum-infected red blood cells with uninfected red blood cells enhances microvascular obstruction under flow conditions. *Blood,* 78**,** 812-819.

KAUSAR, M. W., MOEED, K., ASIF, N., RIZWI, F. & RAZA, S. 2010. Correlation of bilirubin with liver enzymes in patients of falciparum malaria. *International Journal of Pathology,* 8**,** 63-67.

KAVANAUGH, A., ADAMS-HUET, B., JAIN, R., DENKE, M. & MCFARLIN, J. 1997. Hydroxychloroquine Effects on Lipoprotein Profiles (the HELP trial): A Double-Blind, Randomized, Placebo-Controlled, Pilot Study In Patients With Systemic Lupus Erythematosus. *Journal of Clinical Rheumatology,* 3**,** 3-8.

KNUTSON, V. P., RONNETT, G. V. & LANE, M. D. 1985. The effects of cycloheximide and chloroquine on insulin receptor metabolism. Differential effects on receptor cycling and inactivation and insulin degradation. *The Journal of Biological Chemistry,* 260**,** 14180-14188.

KONGPATANAKUL, S., CHATSIRICHAROENKUL, S., KHUHAPINANT, A., ATIPAS, S. & KAEWKUNGWAL, J. 2009. Comparative study of dihydroartemisinin and artesunate safety in healthy Thai volunteers. *International Journal of Therapeutics,* 47(9)**,** 579–586.

KREMSNER, P. G. & KRISHNA, S. 2004. Antimalarial combinations. *Lancet* 364**,** 285-294.

KRISHNA, S., TAYLOR, A. M., SUPANARANOND, W., PUKRITTAYAKAMEE, S., TER KUILE, F., TAWFIQ, K. M., HOLLOWAY, P. A. & WHITE, N. J. 1999. Thiamine deficiency and malaria in adults from southeast Asia. *Lancet,* 353**,** 546-9.

KWITEROVICH, P. O. 2000. The Metabolic Pathways of High-Density Lipoprotein, Low-Density Lipoprotein, and Triglycerides: A Current Review. *American Journal of Cardiology®*86**,** 5L-10L.

LAMARCHE, B., RASHID, S. & LEWIS, G. F. 1999. HDL metabolism in hypertriglyceridemic states: an overview. *Clinica Chimica Acta* 286**,** 145-161.

LANGER, R. 2000. Biomaterials in drug delivery and tissue engineering: one laboratory's experience. *Accounts of Chemical Research,* 33**,** 94-101.

LARRUCEA, E., ARELLANO, A., SANTOYO, S. & YGARTUA, P. 2001. Combined effects of oleic acid and propylene glycol on the percutaneous penetration of tenoxicam and its retention in the skin. *European Jouranl of Phamaceutics and Biopharamaceutics* 52**,** 113-119.

LEDERMAN, E. R., MAGUIRE, J. D., SUMAWINATA, I. W., CHAND, K., ELYAZAR, I., ESTIANA, L., SISMADI, P., BANGS, M. J. & BAIRD, J. K. 2006. Combined chloroquine, sulfadoxine/pyrimethamine and primaquine against Plasmodium falciparum in Central Java, Indonesia. *Malaria Journal,* 5**,** 1-10.

LIBBY, P. 2001. Managing the Risk of Atherosclerosis: The Role of High-Density Lipoprotein. *American Journal of Cardiology®*88**,** 3N-8N.

LIBBY, P., RIDKER, P. M. & MASERI, A. 2000. Inflammation and Atherosclerosis. *Circulation* 105**,** 1135-1143.

LINK, J. J., ROHATGI, A. & DE LEMOS, J. A. 2007. HDL Cholesterol: Physiology, Pathophysiology, and Management. *Current Problems in Cardiology,* 32**,** 268-314.

LIRON, Z. & COHEN, S. 1984a. Percutaneous absorption of alkanoic acids I: A study of operational conditions. *Journal of Pharmaceutical Sciences,* 73**,** 534-7.

LIRON, Z. & COHEN, S. 1984b. Percutaneous absorption of alkanoic acids II: Application of regular solution theory. *Journal of Pharmaceutical Science*s, 73**,** 538-42.

LOFGREN, C., GUILLOTIN, S. & HERMANSSON, A. 2006. Microstructure and Kinetic Rheological Behavior of Amidated and Nonamidated LM Pectin Gels. *Biomacromolecules,* 7**,** 114-121.

LOOAREESUWAN, S., WHITE, N. J., CHANTHAVANICH, P., EDWARDS, G., NICHOLL, D. D., BUNCH, C. & WARRELL, D. A. 1986. Cardiovascular toxicity and distribution kinetics of intravenous chloroquine. *British Journal of Clinical Pharmacology,* 22, 31-6.

MACKINTOSH, C. L., J.G.BEESON & MARSH, K. 2004. Clinical features and pathogenesis of severe malaria. *TRENDS in Parasitology* 20.

MANAN, J. A., ALI, H. & LAL, M. 2006. Acute Renal Failure Associated with Malaria. *J Ayub Med Coll Abbottabad,* 18**,** 47.

MARQUARDT, K. & ALBERTSON, T. E. 2001. Treatment of Hydroxychloroquine Overdose. *American Journal of Emergency Medicine,* 19**,** 420-424.

MATIA-MERINO, L., LAU, K. & DICKINSON, E. 2004. Effects of low-methoxyl amidated pectin and ionic calcium on rheology and microstructure of acid-induced sodium caseinate gels. *Food Hydrocolloids,* 18**,** 271–281.

MCCHESNEY, E. W., BANKS, W. F., JR. & FABIAN, R. J. 1967. Tissue distribution of chloroquine, hydroxychloroquine, and desethylchloroquine in the rat. *Toxicology and Applied Pharmacology,* 10**,** 501-13.

MEGWA, S. A., CROSS, S. E., BENSON, H. A. & ROBERTS, M. S. 2000a. Ion-pair formation as a strategy to enhance topical delivery of salicylic acid. *Journal of Pharmacy and Pharmacology,* 52**,** 919-28.

MEGWA, S. A., CROSS, S. E., WHITEHOUSE, M. W., BENSON, H. A. & ROBERTS, M. S. 2000b. Effect of ion pairing with alkylamines on the in-vitro dermal penetration and local tissue disposition of salicylates. *Journal of Pharmacy and Pharmacology,* 52**,** 929-40.

MENENDEZ, C., FLEMING, A. F. & ALONSO, P. L. 2000. Malaria-related Anaemia. *Parasitology Today,* 16(11)**,** 469-476.

MENON, G. K. 2002. New insights into skin structure: scratching the surface. *Advanced Drug Delivery Reviews,* 54 Suppl 1**,** S3-17.

MENON, G. K., BOMMANNAN, D. B. & ELIAS, P. M. 1994. High-frequency sonophoresis: permeation pathways and structural basis for enhanced permeability. *Skin Pharmacology,* 7**,** 130-9.

MESSANT, I., JÉRÉMIE, N., LENFANT, F. & FREYSZ, M. 2004. Massive chloroquine intoxication: importance of early treatment and pre-hospital treatment. *Resuscitation* 60**,** 343-346.

MISHRA, M. K., RAY, D. & BARIK, B. B. 2009. Microcapsules and transdermal patch: a comparative approach for improved delivery of antidiabetic drug. *AAPS PharmSciTech,* 10**,** 928-34.

MITRAGOTRI, S. 2000. Synergistic effect of enhancers for transdermal drug delivery. *Pharmaceutical Research*17**,** 1354-9.

MITRAGOTRI, S. & KOST, J. 2000. Low-frequency sonophoresis: a noninvasive method of drug delivery and diagnostics. *Biotechnology Progress,* 16**,** 488-92.

MOLYNEUX, D. H., FLOYD, K., BARNISH, G. & FEVRE, E. M. 1999. Transmission control and drug resistance in malaria: a crucial interaction. *Parasitology Today,* 15**,** 238-40.

MONTI, D., CHETONI, P., BURGALASSI, S., NAJARRO, M., SAETTONE, M. F. & BOLDRINI, E. 2002. Effect of different terpene-containing essential oils on permeation of estradiol through hairless mouse skin. *International Journal of Pharmaceutics,* 237**,** 209-14.

MORIMOTO, H., WADA, Y., SEKI, T. & SUGIBAYASHI, K. 2002. In vitro skin permeation of morphine hydrochloride during the finite application of penetration-enhancing system containing water, ethanol and L-menthol. *Biological and Pharmaceutical Bulletin,* 25**,** 134–136.

MORROW, D. I. J., MCCARRON, P. A., WOOLFSON, A. D. & DONNELLY, R. F. 2007. Innovative Strategies for Enhancing Topical and Transdermal Drug Delivery. *The Open Drug Delivery Journal,* 1**,** 36-59.

MOSER, K., KRIWET, K., FROEHLICH, C., KALIA, Y. N. & GUY, R. H. 2001. Supersaturation: enhancement of skin penetration and permeation of a lipophilic drug. *Pharmaceutical Research,* 18**,** 1006-11.

MUBAGWA, K. & ADLER, J. 1988. Muscarinic antagonist action of clinical doses of chloroquine in healthy volunteers. *Journal of the Autonomic Nervous System.,* 24(1-2)**,** 147-55.

MULENGA, M., MALUNGA, F., BENNETT, S., THUMA, P. E., SHULMAN, C., FIELDING, K., ALLOUECHE, A. & GREENWOOD, B. M. 2006. A randomised, double-blind, placebocontrolled trial of atovaquone–proguanil vs. sulphadoxine–pyrimethamine in the treatment of malarial anaemia in Zambian children. *Tropical Medicine and International Health,* 11 (11)**,** 1643–1652.

MUNJERI, O., HODZA, P., OSIM, E. E. & MUSABAYANE, C. T. 1998. An investigation into the suitability of amidated pectin hydrogel beads as a delivery matrix for chloroquine. *Journal of Pharmaceutical Sciences,* 87, 905-8.

MUNRO, R., MORRISON, E., MCDONALD, A. G., HUNTER, J. A., MADHOK, R. & CAPELL, H. A. 1997. Effect of disease modifying agents on the lipid profiles of patients with rheumatoid arthritis. *Annals of the Rheumatic Diseases,* 56**,** 374-7.

MUSABAYANE, C. T., COOPER, R. G., RAO, P. V. & BALMENT, R. J. 2000a. Effects of ethanol on the changes in renal fluid and electrolyte handling and kidney morphology induced by long-term chloroquine administration to rats. *Alcohol,* 22**,** 129-38.

MUSABAYANE, C. T., FORSLING, M. L. & BALMENT, R. J. 1997. Arginine vasopressin increases renal sodium excretion in the anesthetized rat through V1 receptors. *Renal Failure,* 19**,** 23-32.

MUSABAYANE, C. T., GONDWE, M., KAMADYAAPA, D. R., CHUTURGOON, A. A. & OJEWOLE, J. A. 2007. Effects of Ficus thonningii (Blume) [Morarceae] stem-bark ethanolic extract on blood glucose, cardiovascular and kidney functions of rats, and on kidney cell lines of the proximal (LLC-PK1) and distal tubules (MDBK). *Renal Failure,* 29**,** 389-97.

MUSABAYANE, C. T., MUNJERI, O., BWITITI, P. & OSIM, E. E. 2000b. Orally administered, insulin-loaded amidated pectin hydrogel beads sustain plasma concentrations of insulin in streptozotocin-diabetic rats. *Journal of Endocrinology,* 164**,** 1-6.

MUSABAYANE, C. T., MUNJERI, O. & MATAVIRE, T. P. 2003. Transdermal delivery of chloroquine by amidated pectin hydrogel matrix patch in the rat. *Renal Failure,* 25**,** 525-34.

MUSABAYANE, C. T., MUSVIBE, A., WENYIKA, J., MUNJERI, O. & OSIM, E. E. 1999. Chloroquine influences renal function in rural Zimbabweans with acute transient fever. *Renal Failure,* 21**,** 189-97.

MUSABAYANE, C. T., NDHLOVU, C. E. & BALMENT, R. J. 1994. The effects of oral chloroquine administration on kidney function. *Renal Failure,* 16**,** 221-8.

MUSABAYANE, C. T., NDHLOVU, C. E., MAMUTSE, G., BWITITI, P. & BALMENT, R. J. 1993. Acute chloroquine administration increases renal sodium excretion. *Journal of Tropical Medicine and Hygiene,* 96**,** 305-10.

MUSABAYANE, C. T., WARGENT, E. T. & BALMENT, R. J. 2000c. Chloroquine inhibits arginine vasopressin production in isolated rat inner medullary segments induced cAMP collecting duct. *Renal Failure,* 22**,** 27-37.

MUSABAYANE, C. T., WINDLE, R. J., FORSLING, M. L. & BALMENT, R. J. 1996. Arginine vasopressin mediates the chloroquine induced increase in renal sodium excretion. *Tropical Medicine and International Health,* 1**,** 542-50.

MUTURI, E. J., BURGESS, P. & NOVAK, R. J. 2008. Malaria Vector Management: Where Have We Come From and Where Are We Headed? *The American Society of Tropical Medicine and Hygiene,* 78, 536-537.

NATARAJAN, P., RAY, K. K. & CANNON, C. P. 2010. High-Density Lipoprotein and Coronary Heart Disease. Current and Future Therapies. *Journal of the American College of Cardiology,* 55**,** 1283-1299.

NAQVI, R., AHMAD, E., AKHTAR, F., NAQVI, A. & RIZVI, A. 2003. Outcome in severe acute renal failure associated with malaria. *Nephrology Dialysis Transplantation,* 18**,** 1820-3.

NARAYANAN, S. & APPLETON, H. D. 1980. Creatinine: a review. *Clinical Chemistry,* 26**,** 1119-26.

NAVAB, M., BERLINER, J. A., WATSON, A. D., HAMA, S. Y., TERRITO, M. C., LUSIS, A. J., SHIH, D. M., VAN LENTEN, B. J., FRANK, J. S., DEMER, L. L., EDWARDS, P. A. & FOGELMAN, A. M. 1996. The Yin and Yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff Memorial Lecture. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 16**,** 831-42.

NEWTON, C. R., KIRKHAM, F. J., WINSTANLEY, P. A., PASVOL, G., PESHU, N., WARRELL, D. A. & MARSH, K. 1991. Intracranial pressure in African children with cerebral malaria. *Lancet,* 337**,** 573-6.

NEWTON, C. R., PESHU, N., KENDALL, B., KIRKHAM, F. J., SOWUNMI, A., WARUIRU, C., MWANGI, I., MURPHY, S. A. & MARSH, K. 1994. Brain swelling and ischaemia in Kenyans with cerebral malaria. *Archives of Disease in Childhood,* 70**,** 281-7.

NGAHA, E. O. 1982. Some biochemical changes in the rat during repeated chloroquine administration. *Toxicology Letters,* 10**,** 145-9.

NGUBANE, P. S., MASOLA, B. & MUSABAYANE, C. T. 2011. The effects of Syzygium aromaticum-derived oleanolic acid on glycogenic enzymes in streptozotocin-induced diabetic rats. *Renal Failure,* 33**,** 434-9.

NOSTEN, F. & WHITE, N. J. 2007. Artemisinin-based combination treatment of falciparum malaria. *American Journal of Tropical Medicine and Hygiene,* 77, 6**,** 181–192.

NWANYANWU, O. C., ZIBA, C., KAZEMBE, P., CHITSULO, L., WIRIMA, J. J., KUMWENDA, N. & REDD, S. C. 1996. Efficacy of sulphadoxine/pyrimethamine for Plasmodium falciparum malaria in Malawian children under five years of age. *Tropical Medicine and International Health,* 1**,** 231-5.

OBI, F. I., MADUKA, H. C. C. & MAMZA, Y. P. 2003. Effects of chloroquine on blood glucose and cholesterol levels in alloxan-induced diabetic rabbits. *Journal of Biological Sciences,* 3, 10**,** 875-881.

OGBONNA, A. & UNEKE, C. 2008. Artemisinin-based combination therapy for uncomplicated malaria in sub-Saharan Africa: the efficacy, safety, resistance and policy implementation since Abuja 2000. *Transactions of the Royal Society of Tropical Medicine & Hygiene,* 102, 7**,** 621–627.

OGBADOYI, E. O. & TSADO, R. D. 2009. Renal and Hepatic Dysfunction in Malaria Patients in Minna, North Central Nigeria. *Online Journal of Health and Allied Sciences,* 8**,** 1-6.

OLLIARO, P. L. & TAYLOR, W. R. J. 2004. Developing artemisinin based drug combinations for the treatment of drug resistant falciparum malaria. *Journal of Postgraduate Medicine,* 50**,** 41-44.

ONGPIPATTANAKUL, B., BURNETTE, R. R., POTTS, R. O. & FRANCOEUR, M. L. 1991. Evidence that oleic acid exists in a separate phase within stratum corneum lipids. *Pharmaceutical Research,* 8**,** 350-4.

ONYESOM, I. & AGHO, J. E. 2011. Changes in serum glucose and triacylglycerol levels induced by the co-administration of two different types of antimalarial drugs among some Plasmodium falciparum malarial patients in Edo-delta region of Nigeria. *Asian Journal of Scientific Research,* 4**,** 78-83.

PADULA, C., NICOLI, S., AVERSA, V., COLOMBO, P., FALSON, F., PIROT, F. & SANTI, P. 2007. Bioadhesive film for dermal and transdermal drug delivery. *European Journal of Dermatology,* 17**,** 309-12.

PANDEY, A. V., BISHT, H., BABBARWAL, V. K., SRIVASTAVA, J., PANDEY, K. & CHAUHAN, V. S. 2001. Mechanism of malarial haem detoxification inhibition by chloroquine. *Biochemical Journal. ,* 355**,** 333-338.

PATHAN, I. B. & SETTY, C. M. 2009. Chemical Penetration Enhancers for Transdermal Drug Delivery Systems. *Tropical Journal of Pharmaceutical Research,* 8, 2**,** 173-179.

PETRI, M., LAKATTA, C., MAGDER, L. & GOLDMAN, D. 1994. Effect of prednisone and hydroxychloroquine on coronary artery disease risk factors in systemic lupus erythematosus: a longitudinal data analysis. *American Journal of Medicine,* 96**,** 254-9.

PHILLIPS, R. E. 1989. Hypoglycaemia is an important complication of falciparum malaria. *Quartely Journal of Medicine,* 71**,** 477-83.

PHILLIPS, R. E., LOOAREESUWAN, S., WHITE, N. J., CHANTHAVANICH, P., KARBWANG, J., SUPANARANOND, W., TURNER, R. C. & WARRELL, D. A. 1986. Hypoglycaemia and antimalarial drugs: quinidine and release of insulin. *British Medical Journal (Clinical research ed.),* 292**,** 1319-21.

POTTS, R. O. & FRANCOEUR, M. L. 1990. Lipid biophysics of water loss through the skin. *Proceedings of the National Academy of Sciencesof the United States,* 87**,** 3871-3.

POTTS, R. O. & GUY, R. H. 1992. Predicting skin permeability. *Pharmaceutical Research,* 9**,** 663-9.

POWELL, J. 2006. Skin physiology. *Woman's Health Medicine* 3, 3**,** 130-133.

POWELL, J. & SOON, C. 2002. Physiology of the Skin. *Surgery,* 1**,** I-VI.

PRAKASH, J., GUPTA, A., KUMAR, O., ROUT, S. B., MALHOTRA, V. & SRIVASTAVA, P. K. 1996. Acute renal failure in falciparum malaria--increasing prevalence in some areas of India--a need for awareness. *Nephrology Dialysis Transplantation,,* 11**,** 2414-6.

PRAKASH, J., SINGH, A. K., GUJRATI, S. & MAHESHWARI, A. 2002. Acute renal failure in Malaria: Changing trends. *Indian Journal of Nephrology,* 12**,** 113-117.

PRAUSNITZ, M. R., GIMM, J. A., GUY, R. H., LANGER, R., WEAVER, J. C. & CULLANDER, C. 1996. Imaging regions of transport across human stratum corneum during high-voltage and low-voltage exposures. *Journal of Pharmaceutical Sciences,* 85**,** 1363-70.

PRAUSNITZ, M. R. & LANGER, R. 2008. Transdermal Drug Delivery. *Natural Biotechnology,* 26**,** 1261-1268.

PROJEAN, D., BAUNE, B., FARINOTTI, R., FLINOIS, J. P., BEAUNE, P., TABURET, A. M. & DUCHARME, J. 2003. In vitro metabolism of chloroquine: identification of CYP2C8, CYP3A4, and CYP2D6 as the main isoforms catalyzing N-desethylchloroquine formation. *Drug Metabolism and Disposition* 31**,** 748-754.

PUSSARD, E. & VERDIER, F. 1994. Antimalarial 4-aminoquinolines: mode of action and pharmacokinetics. *Fundamental & Clinical Pharmacology,* 8**,** 1-17.

RADER, D. J. 2002. High-Density Lipoproteins and Atherosclerosis. *American Journal of Cardiology®,* 90**,** 62i–70i.

RAHMAN, P., GLADMAN, D. D., UROWITZ, M. B., YUEN, K., HALLETT, D. & BRUCE, I. N. 1999. The cholesterol lowering effect of antimalarial drugs is enhanced in patients with lupus taking corticosteroid drugs. *Journal of Rheumatology,* 26**,** 325-30.

RAYNES, K. 1999. Bisquinoline antimalarials their role in malaria chemotherapy. *International Journal for Parasitology* 29**,** 367-379.

RHEE, Y. S., KWON, S. Y., PARK, C. W., CHOI, N. Y., BYUN, W. J., CHI, S. C. & PARK, E. S. 2008. Characterization of monolithic matrix patch system containing tulobuterol. *Archives of Pharmacal Research,* 31**,** 1029-34.

RIDLEY, B. L., O'NEILL, M. A. & MOHNEN, D. 2001. Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry,* 57**,** 929-67.

RIOU, B., LECARPENTIER, Y., BARRIOT, P. & VIARS, P. 1989. Diazepam does not improve the mechanical performance of rat cardiac papillary muscle exposed to chloroquine in vitro. *Intensive Care Medicine,* 15**,** 390–95.

RIOU, B., RIMAILHO, A., GALLIOT, M., BOURDON, R. & HUET, Y. 1988. Protective cardiovascular eff ects of diazepam in experimental acute chloroquine poisoning. *Intensive Care Medicine,* 14**,** 610–16.

RIVIEREA, J. E. & PAPICH, M. G. 2001. Potential and problems of developing transdermal patches for veterinary applications. *Advanced Drug Delivery Reviews,* 50**,** 175–203.

ROSS, R. 1999. Atherosclerosis--an inflammatory disease. *New England Journal of Medicine,* 340**,** 115-26.

ROY, S. D., GUTIERREZ, M., FLYNN, G. L. & CLEARY, G. W. 1996. Controlled transdermal delivery of fentanyl: characterizations of pressure-sensitive adhesives for matrix patch design. *Journal of Pharmaceutical Sciences,* 85**,** 491-5.

RUBINSTEIN, A., RADAI, R., EZRA, M., PATHAK, S. & ROKEM, J. S. 1993. In vitro evaluation of calcium pectinate: a potential colon-specific drug delivery carrier. *Pharmaceutical Research,* 10**,** 258-63.

RUBIO, J. M., BUHIGAS, I., SUBIRATS, M., BAQUERO, M., PUENTE, S. & BENITO, A. 2001. Limited level of accuracy provided by available rapid diagnosis tests for malaria enhances the need for PCR-based reference laboratories. *Journal of Clinical Microbiology.,* 39, 7**,** 2736-2737.

SANCHEZ-CHAPULA, J. A., SALINAS-STEFANON, E., TORRES-JACOMW, J., BENAVIDES, D. E. & NAVARRO-POLANCO, R. A. 2001. Blockade of currents by the antimalarial drug chloroquine in feline ventricular myocytes. *Journal of Pharmacology and Experimental Therapeutics,* 297**,** 437–445.

SANGUINETTI, M. C. & JURKIEWICZ, N. K. 1990. Two components of cardiac delayed rectifier K+ current. Differential sensitivity to block by class III antiarrhythmic agents. *Journal of General Physiology,* 96**,** 195-215.

SARVEIYA, V., TEMPLETON, J. F. & BENSON, H. A. 2004. Ion-pairs of ibuprofen: increased membrane diffusion. *Journal of Pharmacy and Pharmacology,* 56**,** 717-24.

SCHEUPLEIN, R. J. & BLANK, I. H. 1973. Mechanism of percutaneous absorption. IV. Penetration of nonelectrolytes (alcohols) from aqueous solutions and from pure liquids. *Journal of Investigative Dermatology,* 60**,** 286-96.

SCHWENKE, D. C. & CAREW, T. E. 1989. Initiation of atherosclerotic lesions in cholesterol-fed rabbits. II. Selective retention of LDL vs. selective increases in LDL permeability in susceptible sites of arteries. *Arteriosclerosis,* 9**,** 908-18.

SHAH, R. R. 2005. Drugs, QTc interval prolongation and final ICH E14 guideline : an important milestone with challenges ahead. *Drug Safety,* 28**,** 1009-28.

SHAHIWALA, A. & MISRA, A. 2002. Studies in topical application of niosomally entrapped Nimesulide. *Journal of Pharmaceutical Sciences,* 5**,** 220-5.

SINTOV, A., ZE'EVI, A., UZAN, R. & NYSKA, A. 1999. Influence of pharmaceutical gel vehicles containing oleic acid/sodium oleate combinations on hairless mouse skin, a histological evaluation. *European Journal of Pharmaceutics and Biopharmaceutics,* 47**,** 299-303.

SIRTORI, C. R. 2004. New frontiers in atherosclerosis prevention and treatment: HDL as a novel therapeutic target. *International Congress Series* 1262**,** 9-14.

SLATER, A. F. 1993. Chloroquine: mechanism of drug action and resistance in Plasmodium falciparum. *Pharmacology & Therapeutics,* 57**,** 203-35.

SLOAN, K. B., KOCH, S. A., SIVER, K. G. & FLOWERS, F. P. 1986. Use of solubility parameters of drug and vehicle to predict flux through skin. *Journal of Investigative Dermatology,* 87**,** 244-52.

SMITH, G. L., SHLIPAK, M. G., HAVRANEK, E. P., FOODY, J. M. & MASOUDI, F. A. 2006. Serum urea nitrogen, creatinine and estimators of renal function. *Archives of. Internal. Medicine,* 166**,** 1134-1142.

SMITH, G. D., AMOS, T. A., MAHLER, R. & PETERS, T. J. 1987. Effect of chloroquine on insulin and glucose homeostasis in normal subjects and patients with non-insulin dependant diabetes mellitus. *British Medical Journal (Clinical Research and Education),* 294**,** 465-467.

SNOW, R. 2003. The public health burden of Plasmodium falciparum malaria in Africa: deriving the numbers. *Fogarty International Center/National Institutes of Health. (The Disease Control Priorities Project (DCPP) Working Paper Series No. 11).*

SOFOLA, O. A. 2008. The effects of chloroquine on the electrocardiogram and heart rate in anaesthetized dogs. *Clinical Physiology and Functional Imaging,* 3, 1**,** 75 - 82.

SOFOLA, O. A., OLUDE, I. O. & ADEGOKE, F. 1981. The effect of chronic chloroquine toxicity on blood pressure of rats. *Journal of Tropical Medicine & Hygiene,* 84**,** 249-52.

SONGKRO, S. 2009. An overview of skin penetration enhancers: penetration enhancing activity, skin irritation potential and mechanism of action. *Songklanakarin Journal of Science and Technology.,* 31 (3)**,** 299-321.

SRIAMORNSAK, P. 1998. Preliminary investigation of some polysaccharides as a carrier for cell entrapment. *European Journal of Pharmaceutics and Biopharmaceutics,* 46**,** 233-6.

STOTT, P. W., WILLIAMS, A. C. & BARRY, B. W. 1998. Transdermal delivery from eutectic systems: enhanced permeation of a model drug, ibuprofen. *Journal of Controlled Release,* 50**,** 297-308.

SUPANARANOND, W., DAVIS, T. M. E., PUKRITTAYAKAMEE, S., NAGACHINTA, B. & WHITE, N. J. 1993. Abnormal circulatory control in falciparum malaria:the effects of antimalarial drugs. *European Journal Clinical Pharmacology,* 44**,** 325-329.

TAKAHASHI, K., SAKANO, H., NUMATA, N., KURODA, S. & MIZUNO, N. 2002. Effect of fatty acid diesters on permeation of anti-inflammatory drugs through rat skin. *Drug Development and Industrial Pharmacy,* 28.

TAKEUCHI, H., MANO, S., SAKURAI, T., FURUYA, A., URANO, H. & SUGIBAYASHI, K. 2011. Usefulness of Rat Skin as a Substitute for Human Skin in the in Vitro Skin Permeation Study. *Experimental Animals* 60**,** 373-384.

TAM, L. S., GLADMAN, D. D., HALLETT, D. C., RAHMAN, P. & UROWITZ, M. B. 2000a. Effect of antimalarial agents on the fasting lipid profile in systemic lupus erythematosus. *Journal of Rheumatology,* 27**,** 2142-5.

TAM, L. S., LI, E. K., LAM, C. W. & TOMLINSON, B. 2000b. Hydroxychloroquine has no significant effect on lipids and apolipoproteins in Chinese systemic lupus erythematosus patients with mild or inactive disease. *Lupus,* 9**,** 413-6.

THIELE, J. J. & EKANAYAKE-MUDIYANSELAGE, S. 2007. Vitamin E in human skin: organ-specific physiology and considerations for its use in dermatology. *Molecular Aspects of Medicine,* 28**,** 646-67.

THIEN, H. V., KAGER, P. A. & SAUERWEIN, H. P. 2006. Hypoglycemia in falciparum malaria: is fasting an unrecognized and insufficiently emphasized risk factor? *Trends in Parasitology,* 22**,** 410-5.

TOTH, P. P. 2005. The "Good Cholesterol": High-Density Lipoprotein. *Circulation* 111**,** e89-e91.

TOUITOU, E., DAYAN, N., BERGELSON, L., GODIN, B. & ELIAZ, M. 2000. Ethosomes - novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *Journal of Controlled Release,* 65**,** 403-18.

TOUITOU, E., GODIN, B., DAYAN, N., WEISS, C., PILIPONSKY, A. & LEVI-SCHAFFER, F. 2001. Intracellular delivery mediated by an ethosomal carrier. *Biomaterials,* 22**,** 3053-9.

TOUITOU, E., JUNGINGER, H. E., WEINER, N. D., NAGAI, T. & MEZEI, M. 1994. Liposomes as carriers for topical and transdermal delivery. *Journal of Pharmaceutical Sciences,* 83**,** 1189-203.

TOUITOU, E., MEIDAN, V. M. & HORWITZ, E. 1998. Methods for quantitative determination of drug localized in the skin. *Journal of Controlled Release,* 56**,** 7-21.

TRIVEDI, J. S., KRILL, S. L. & FORT, J. J. 1995. Vitamin E as a human skin penetration enhancer. *European Journal of Pharmaceutical Sciences,* 3**,** 241-243.

TROMMER, H. & NEUBERT, R. H. 2006. Overcoming the stratum corneum: the modulation of skin penetration. A review. *Skin pharmacology and physiology,* 19**,** 106-21.

TULPULE, A. & KRISHNASWAMY, K. 1982. Effect of food on bioavailability of chloroquine. *European Journal Clinical Pharmacology,* 23**,** 271-3.

TUTEJA, R. 2007. Malaria - an overview. *FEBS Journal,* 274**,** 4670-9.

URSING, J., KOFOED, P., RODRIGUES, A., BERGQVIST, Y. & ROMBO, L. 2009. Chloroquine Is Grossly Overdosed and Overused but Well Tolerated in Guinea-Bissau. *Antimicrobial Agents and Chemotherapy,* 53**,** 180 - 185.

UYEMURA, S. A., LUO, S., MORENO, S. N. & DOCAMPO, R. 2000. Oxidative phosphorylation, Ca^{2+} transport, and fatty acid-induced uncoupling in malaria parasites mitochondria. *Journal of Biological Chemistry,* 275**,** 9709-15.

VALENTA, C., SIMAN, U., KRATZEL, M. & HADGRAFT, J. 2000. The dermal delivery of lignocaine: influence of ion pairing. *International Journal of Pharmaceutics,* 197**,** 77-85.

VENKATRAMAN, S. & GALE, R. 1998. Skin adhesives and skin adhesion. 1. Transdermal drug delivery systems. *Biomaterials,* 19**,** 1119-36.

VENUS, M., WATERMAN, J. & MCNAB, I. 2010. Basic physiology of the skin. *Surgery,* 28, 10**,** 469-472.

WALKER, O., DAWODU, A. H., ADEYOKUNNU, A. A., SALAKO, L. A. & ALVAN, G. 1983. Plasma chloroquine and desethylchloroquine concentrations in children during and after chloroquine treatment for malaria. *British Journal of Clinical Pharmacology,* 16**,** 701-5.

WALLACE, D. J., METZGER, A. L., STECHER, V. J., TURNBULL, B. A. & KERN, P. A. 1990. Cholesterol-lowering effect of hydroxychloroquine in patients with rheumatic disease: reversal of deleterious effects of steroids on lipids. *American Journal of Medicine,* 89**,** 322-6.

WARHURST, D. 2001. New developments: Chloroquine-resistance in Plasmodium falciparum. *Drug Resistance Updates,* 4**,** 141-144.

WARNICK, G. R. & WOOD, P. D. 1995 National cholesterol education program recomandations for measurement of high density lipoprotein cholesterol: executive summary. *Clinical chemistry,* 41**,** 1427-1433.

WETSTEIN, J. C. F. M., DE VRIES, P. J., OOSTERHUIS, B. & VAN BOXTEL, C. J. 1995. The pharmacokinetics of three multiple dose regimens of chloroquine: implications for malaria chemoprophylaxis. *British Journal of Clinical Pharmacology,* 39**,** 696-699.

WELLER, R. E., COLLINS, W. E., BUSCHBOM, R. L., MALAGA, C. A. & RAGAN, H. A. 1992. Impaired renal function in owl monkeys (Aotus nancymai) infected with Plasmodium falciparum. *Mem Inst Oswaldo Cruz,* 87 Suppl 3**,** 435-42.

WHITE, N. J. 1992. Antimalarial pharmacokinetics and treatment regimens. *British Journal of Clinical Pharmacology. ,* 34**,** 1-10.

WHITE, N. J. 2008. The role of anti-malarial drugs in eliminating malaria. *Malaria Journal* 7**,** 1-6.

WHITE, N. J., NOSTEN, F., LOOAREESUWAN, S., WATKINS, W. M., MARSH, K., SNOW, R. W., KOKWARO, G., OUMA, J., HIEN, T. T., MOLYNEUX, M. E., TAYLOR, T. E., NEWBOLD, C. I., RUEBUSH, T. K., 2ND, DANIS, M., GREENWOOD, B. M., ANDERSON, R. M. & OLLIARO, P. 1999. Averting a malaria disaster. *Lancet,* 353**,** 1965-7.

WHITTY, C. J. M., CHANDLER, C., ANSAH, E., LESLIE, T. & STAEDKE, S. G. 2008. Deployment of ACT antimalarials for treatment of malaria: challenges and opportunities. *Malaria Journal* 7**,** 1-7.

WILLIAMS, A. C. & BARRY, B. W. 1991. Terpenes and the lipid-protein-partitioning theory of skin penetration enhancement. *Pharmaceutical Research,* 8**,** 17-24.

WILLIAMS, H. A., CAUSER, L., METTA, E., MALILA, A., O'REILLY, T., ABDULLA, S., KACHUR, S. P. & BLOLAND, P. B. 2008. Dispensary level pilot implementation of rapid diagnostic tests: an evaluation of RDT acceptance and usage by providers and patients– Tanzania 2005. *Malaria Journal,* 7**,** 239.

WINSTANLEY, P. A. 2000. Chemotherapy for Falciparum Malaria: The Armoury, the Problems and the Prospects. *Parasitology Today,* 16**,** 146-153.

WOKOVICH, A. M., PRODDUTURI, S., DOUB, W. H., HUSSAIN, A. S. & BUHSE, L. F. 2006. Transdermal drug delivery system (TDDS) adhesion as a critical safety, efficacy and quality attribute. *European Journal of Pharmaceutics and Biopharmaceutics,* 64**,** 1-8.

World Health Organization, 2006. Guidelines for the treatment of malaria (1st ed.). Report no WHO/HTM/MAL/2006.1108. World Health Organization, Geneva.

World Health Organization, 2008. World malaria report WHO/HTM/GMP/2008.1. WHO, Geneva.

YAP, G. & STEVENSON, M. M. 1994. Inhibition of In Vitro Erythropoiesis by Soluble Mediators in Plasmodium chabaudi AS Malaria: Lack of a Major Role for Interleukin 1, Tumor Necrosis Factor Alpha, and Gamma Interferon. *Infection and Immunity* 62**,** 357-362.

YAYON, A., CABANTCHIK, Z. I. & GINSBURG, H. 1984. Identification of the acidic compartment of Plasmodium falciparuminfected human erythrocytes as the target of the antimalarial drug chloroquine. *The EMBO Journal,* 3**,** 2695 - 2700.

YAYON, A., CABANTCHIK, Z. I. & GINSBURG, H. 1985. Susceptibility of human malaria parasites to chloroquine is pH dependent. *Proceedings of the National Academy of Sciences of the United States,* 82**,** 2784-8.

YAYON, A. & GINSBURG, H. 1983. Chloroquine inhibits the degradation of endocytic vesicles in human malaria parasites. *Cell Biology International Reports,* 7**,** 895.

YEUNG, S., PONGTAVORNPINYO, W., HASTINGS, I. M., MILLS, A. J. & WHITE, N. J. 2004. Antimalarial Drug Resistance, Artemisinin-Based Combination Therapy, and the Contribution of Modelling to elucidating Policy Choices. *The American Society of Tropical Medicine and Hygiene,* 71**,** 179-186.

UNIVERSITY OF KWAZULU-NATAL

02 December 2008

Reference: 018/09/Animal

Ms P Murambiwa
PhD Human Physiology Student
Medical Sciences
University of KwaZulu-Natal
WESTVILLE

Dear Ms Murambiwa

Ethical Approval of Research Project using Animals

I have pleasure in informing you that on recommendation of the review panel, the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for 2009 on the following project:

"Effects of novel chloroquine formulations on renal and cardiovascular functions in experimental animal paradigms".

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc   Registrar
       Research Office
       Head of School

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23 October 2009

Reference: 017/10/Animal

Ms P Murambiwa
PhD Human Physiology Student
School of Medical Science
University of KwaZulu-Natal
WESTVILLE CAMPUS

Dear Ms Murambiwa

Renewal: Ethical Approval of Research Project using Animals

I have pleasure in informing you that the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for 2010 on the following project:

"Effects of novel chloroquine formulations on renal and cardiovascular functions in experimental animal paradigms".

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc   Registrar
      Research Office
      Head of School

24 November 2011

Reference: 10/11/Animal

Ms P Murambiwa Prof. WMU Daniels
Discipline of Human Physiology
Medical Sciences
University of KwaZulu-Natal
WESTVILLE CAMPUS

Dear Ms Murambiwa

Renewal: Ethical Approval of Procedures using Animals for Research

I have pleasure in informing you that on recommendation of the review panel, the Animal Ethics Sub-committee of the University Research and Ethics Committee has granted ethical approval for 2011 on the following project:

"Effects of novel chloroquine formulations on renal and cardiovascular functions in experimental animal paradigms".

Yours sincerely

Prof. Theresa HT Coetzer (Chair)
ANIMAL RESEARCH ETHICS COMMITTEE

Cc   Registrar
      Research Office
      Head of School Prof. WMU Daniels (School office copy)
      Supervisor (Prof. CT Musabayane)

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The effects of chloroquine on renal function in male Sprague-Dawley rats

Murambiwa P[1], Masola B[2], Govender T[4], Mukaratirwa S[3] and Musabayane CT[1]

Disciplines of [1]Human Physiology, [2]Biochemistry, [3]Biological Sciences, [4]Pharmacy and Pharmacology, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa.

CHQ remains the most effective and safe therapeutic option for *falciparum* malaria in sub-Saharan Africa countries. CHQ which is slowly eliminated from the body accumulates in distinct areas of the body such as the kidneys to probably affect function. Several reports have indicated adverse CHQ effects on kidney and cardiovascular system and the retina. Accordingly, this study investigated effects of CHQ on renal fluid and electrolyte handling in rats. Malaria was induced in male Sprague Dawley rats by an i.p. injection of *Plasmodium berghei* (10^5 parasites) to examine renal function for a period of 24 days, divided into pre-treatment, treatment and post-treatment periods. Rats treated with deionised water served as controls.The animals were housed individually in metabolic cages. Following confirmation of stable malaria, animals were treated twice daily with CHQ (60mg. kg^{-1}b.wt, p.o.) for 5 days. All data are presented as means ±SEM. The mean total amounts of food and water taken by CHQ treated animals were low in comparison with control animals. CHQ treatment increased urinary Na^+ outputs and decreased the volume of urine voided, but the urinary urea and creatinine outputs were not altered. However, CHQ treatment also elevated plasma K^+ concentrations with concomitant increase in urinary K^+ outputs. The hyperkalaemia may be attributed to haemolysis of red blood cells due to malaria or CHQ treatment. Blood pressure and glomerular filtration rate were not affected by CHQ treatment. It is concluded that CHQ administration induces an increase in K^+ excretion.

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The effects of chloroquine on selected markers of cardiovasular disease in male sprague-dawley rats
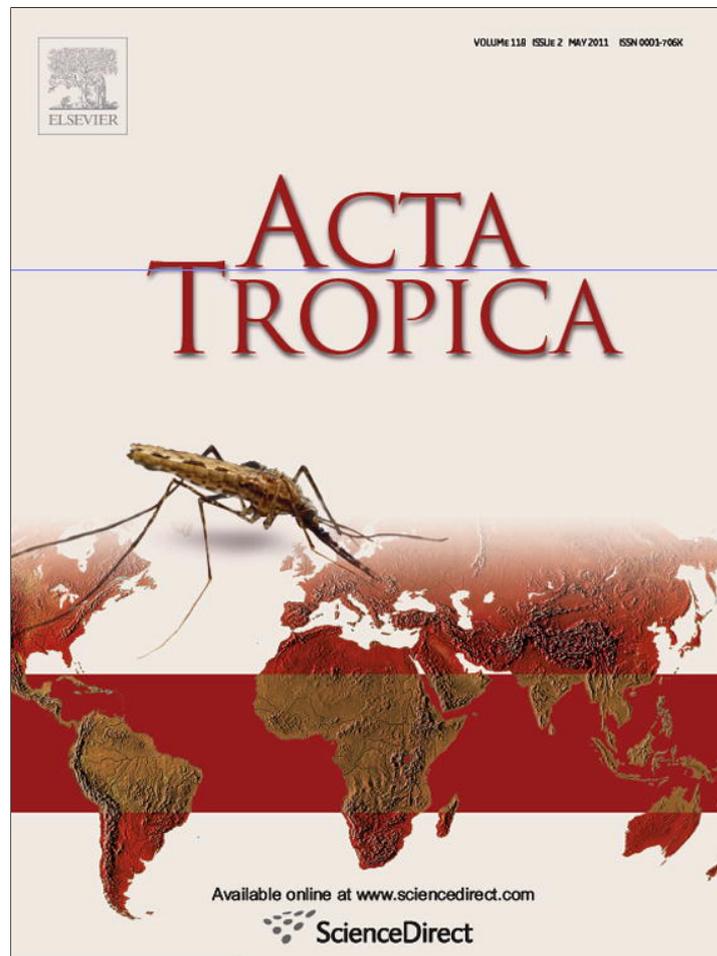
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Impairment of cardiovascular function has been ascribed to *P. falciparum* infection or chloroquine (CHQ) administration in humans and experimental malaria. To distinguish between the pathophysiological effects of malaria and chloroquine on cardiovascular function, we monitored mean arterial pressure and lipid profiles, indicators of the functional status of the cardiovascular system in non-infected (control) and *Plasmodium berghei* infected male Sprague-Dawley rats. *P. berghei* infection is an experimental model of human malaria. Malaria was induced by an i.p. injection of *P berghei* (10^5 parasites). Rats treated with deionised water served as controls. Following confirmation of stable malaria status, animals were treated twice daily, eight hours apart with CHQ (60 mg kg^{-1} b.wt, p.o.) for 5 consecutive days. Blood pressure was measured by a tail cuff method at 09h00 every third day throughout the 21 day experimental period. Animals were euthanized and blood collected on days 7; 14; and 21 post *P. berghei* infection, followed by measurement of plasma Total cholesterol, High Density Lipoprotein-cholesterol (HDL-c) and Low Density Lipoprotein-cholesterol (LDL-c). Mean arterial pressure was not significantly altered by infection or CHQ treatment ($P > 0.05$). However, *P. berghei* infection was associated with a reduction in HDL-c concentration (0.12 ± 0.01 mmol/l,) ($P < 0.05$) in comparison with control animals (0.41 ± 0.06 mmol/l), suggestive of a derangement in the lipid status. On the other hand, *P. berghei* infected animals treated with CHQ were associated with a further reduction in HDL-c to (0.08 ± 0.01 mmol/l) ($P < 0.05$) indicating that the anti-malarial drug alters the lipid status of the animals. Malaria infected animals treated with CHQ were also associated with elevated total cholesterol (2.86 ± 0.35 mmol/l, n=6) ($P < 0.05$) and LDL-c (2.68 ± 0.40 mmol/l), ($P < 0.05$), in comparison to their respective controls (1.21 ± 0.66 mmol/l) and (0.59 ± 0.14 mmol/l), respectively. Reduced HDL-c, elevated total cholesterol and elevated LDL-c concentration signify dyslipidaemia and progression towards atherosclerosis, a process that precedes development of cardiovascular diseases. We can therefore conclude that P. berghei infection may impair cardiovascular function, which is exacerbated by CHQ treatment.

Key words: malaria, cardiovascular function, chloroquine

Anti-malarial drug formulations and novel delivery systems: A review

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ABSTRACT

Artemisinin combination therapies have decreased malaria associated morbidity and mortality in several parts of the world. On the other hand, malaria cases have increased in sub-Saharan Africa largely due to *falciparum* resistance to the most frequently used drugs (chloroquine and sulphadoxine/pyrimethamine (SP) combination). Therapeutic failure has also been attributed in part to adverse effects of anti-malarial drugs and patients' non-compliance due to inconvenient dosing schedules. We consider that formulation and evaluation of novel drug delivery systems is not only less expensive than developing new drugs, but may also improve delivery of anti-malarials at the desired rates. In this review we evaluate the therapeutic efficacy of existing anti-malarial drugs and assess the feasibility of developing novel formulations and delivery systems.

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Contents

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1. Introduction

The elimination of malaria is now considered a realistic goal because of good surveillance and high intervention coverage between 2000 and 2007 which resulted in the reduction of malaria cases and deaths by 50% or more in some countries and regions of African countries (WHO, 2008). Against this background, however, are reports that each year 300-500 million people suffer from acute malaria, and 0.5-2.5 million die of the disease of which 90% are in sub-Saharan Africa (WHO, 2008). Early treatment with effective anti-malarial drugs is the main life-saving intervention but, treatment is threatened by intensification of the growing resistance of *P. falciparum* to drugs that were once effective posing tremendous challenges to malaria control. Indeed, the therapeutic life of widely used anti-malarial drugs, sulphadoxine–pyrimethamine (SP) and amodiaquine also appears to be limited due to an increase in parasite resistance around the world. Given the widespread resistance to available drugs, there is broad consensus to search for effective, safe and affordable new anti-malarial drugs. In this review we focus on the therapeutic efficacy of existing anti-malarial drugs, examine the potential of novel formulation delivery systems of anti-malarial drugs and identify challenges required to optimize malaria therapy. Traditionally, enhancing drug therapy has involved seeking of new chemical entities or incorporation of existing drugs into novel drug delivery systems. It is our view that research should focus on the reformulation of current anti-malarial therapeutic agents for novel delivery routes while developing new drugs.

2. Malaria chemotherapy

Effective malaria chemotherapy aims at treatment of the patient combined with blocking the infectivity of the parasite to the vector by exploiting the differences in metabolism between the *Plasmodium* parasite and the host. Although repeated administration of anti-malarial drugs at sub-therapeutic doses can interrupt parasite transmission, this mode of treatment has been associated with development of drug resistance (Watkins and Masobo, 1993) and is also apparently considered to be the reason for chloroquine (CQ) resistance.

Four *Plasmodium* species, *P. falciparum, P. vivax, P. malariae and P. ovale* cause malaria in humans. *P. falciparum* is the most virulent human malaria parasite due to its rapid rate of reproduction and ability to sequester in small blood vessels (Duong et al., 2004). *P. vivax, P. ovale* and *P. malariae,* rarely cause fatal illness, but *P. knowlesi* may cause death due to its explosive asexual erythrocytic cycle (Cox-Singh et al., 2008). Knowledge of the *Plasmodium* life cycle shown in Fig. 1 is essential for drug interventions studies.

Malaria parasites (sporozoites) inoculated by the female *Anopheles* mosquito (vector) into the vertebrate host blood stream infect the liver cells and mature asexually into merozoites (exoerythrocytic schizogony). The merozoites issuing from the pre-erythrocytic schizogony infect the erythrocytes in blood where merozoites are produced (erythrocytic phase) which differentiate into gametocytes. The erythrocytic phase is responsible for the clinical manifestation of malaria. Mature *Plasmodium* gametocytes are more drug resistant and hence effective malaria treatment should inhibit gametocytogenesis to avert transmission. Drugs with gametocytocidal activity and CQ target this stage of the life cycle. Therefore, artemisinins, primaquine (PQ) and other 8-aminoquinolines amplify the impact of other interventions by inhibiting the sexual stages of *Plasmodium.* The goal of the malaria chemotherapy is to find a drug that has both anti-recrudescence and blood schizocintocidal activity with minimal side-effects.
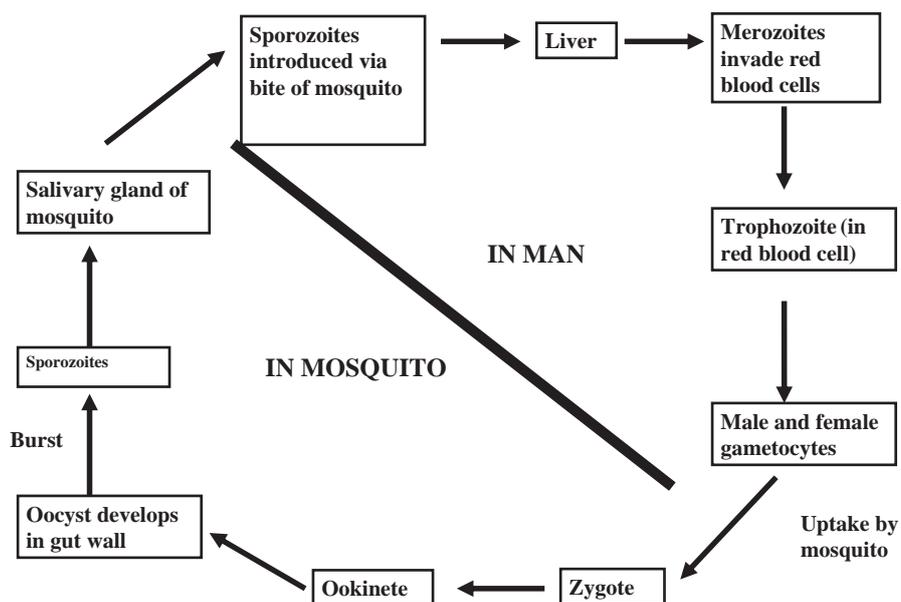

Fig. 1. Illustration of the life cycle of malaria parasites of *Plasmodium* spp.

Table 1
Malaria chemotherapeutic agents.

Drug	Mode of action	Limitations
QN related	Disrupts parasite membrane function resulting in cell lysis and parasite cell autodigestion via production of ferriprotoporphyrin IX	*P falciparum* resistance
9-Quinolinemethanols		
QN	Monoprotic weak base with both blood schizontocidal and gametocytocidal activity	Cardiotoxicity, hypoglycaemic (Ferreras et al., 2007; Alkadi, 2007)
Mefloquine	Diprotic weak base with both blood schizontocidal and gametocytocidal activity	Gastrointestinal disturbances (GI), neuropsychiatry disorders (Alkadi, 2007)
9-Aminoquinolines		
CQ	Blood schizontocidal and gametocytocidal activity via inhibition of the degradation of haeme (which is toxic to the malaria parasite),	Cardiotoxicity, impaired renal function, ocular toxicity (Siqueira-Batista et al., 1998; Alkadi, 2007)
Amodiaquine	Similar to CQ has both blood schizontocidal and gametocytocidal activity	Peripheral neuropathy and haemolytic anaemia (Tafazoli and O'Brien, 2009; Akpalu et al., 2005)
4-Aminoquinoline		
Primaquine	Blood schizontocidal and gametocytocidal activity via inhibition of protein transport	Adverse effects include anaemia and GI disturbances (Shekalaghe et al., 2010; Shanks et al., 2001)
Lumefantrine	Blood schizontocidal and gametocytocidal activities; interferes with detoxifying pathway for the malaria parasite.	No major side effect, except the occurrence of mouth ulcers in some patients (Assefa et al., 2010)
Halofantrine	Mode of action unclear	Cardiaovascular disorders, nausea, vomiting, anorexia and diarrhoea (Olliaro and Bloland, 2001)
Anti-folates	Inhibition of the folate pathway thereby interfering with DNA synthesis	*P. falciparum* resistance
Sulphadoxine–pyrimethamine combination	Blood schizontocidal and gametocytocidal activities	Mental depression, gastrointestinal disorders (Alkadi, 2007)
Proguanil	Dehydrofolate reductase inhibitor with blood schizontocidal and gametocytocidal activity	Dizziness and nausea, acute megaloblastic anaemia (Sirsat and Dasgupta, 1997)
Artemisinin derivatives	Generate free radicals which cause haemolysis and lysis of infected cells	
Artesunate	Blood schizonticide, reduces gametocyte carriage rate	Ataxia, neurotoxicity, decreases reticulocytes, white blood cells and haemoglobin concentration (Kongpatanakul et al., 2009)
Artemether	Blood schizontocidal and gametocytocidal activities	Bradycardia, atrio-ventricular block Rare bradycardia, atrio-ventricular block (Alkadi, 2007)

Plasmodium species exhibit a similar life cycle with only minor variations. For instance a proportion of a form of parasites, hypnozoites from *P. vivax* and *P. ovale* remains dormant in the liver cells before undergoing asexual replication. Relapse can occur any time after the primary attack due to re-activation of a hypnozoites. Whereas relapse usually occurs within the first two or three months of the primary attack malaria due to re-activation of a hypnozoites, there are reports that *P. vivax* hypnozoites can produce clinical infection 1–3 years after the original attack (Garnham et al., 1975; Adak et al., 1998). It should be noted that the therapeutic efficacy of the drugs is limited by factors which include *falciparum* resistance and toxicity (Saussine et al., 2009). A list of some of the currently used anti-malarial drugs given in Table 1 is briefly discussed in the following sections.

3. Artemisinin derivatives

Derivatives of *Artemisia annua* L. (Qinghao, Asteraceae) are considered the cornerstone of the treatment of *falciparum* malaria due to their potency and rapid action (WHO, 2006). Indeed, the introduction of artemisinin-based combination therapy (ACT) interventions decreased morbidity and mortality associated with malaria in several parts of the world (Ogbonna and Uneke, 2008). ACTs in common use include artemether-lumefantrine (Coartem), artesunate-mefloquine and artesunate-amodiaquine. Artemisinin based regimen possess gametocytocidal properties by inhibiting parasite transmission to probably reduce the development of anti-malarial resistance (Nosten and White, 2007). The WHO Expert Consultative Group recommended artemisinin combination therapy (ACT) to combat *falciparum* resistance based on these

properties. Major limitations of ACTs have been ascribed to the imbalance between demand and supply, comparatively high cost, dosing complexity and the lack of clinical experience (Bloland, 2003). Reports of high failure rates associated with ACT therapy along the Thai–Cambodian border (Vijaykadga et al., 2006; Noedl et al., 2008) as well as *in vitro* drug-susceptibility data (Jambou et al., 2005) suggest the possibility of clinical artemisinin resistance. In addition, high doses of artemisinin derivatives have been reported to elicit dose-, time- and route-dependent central nervous system toxicity in laboratory animals (Petras et al., 2000). Interestingly, no serious side effects due to artemisinin derivatives have been reported in human studies to date. We suggest that the discrepancy between animal and human studies can partly be attributed to different routes of administrations.

4. Quinine (QN) related drugs

4.1. Quinine (QN)

Quinine (QN) derived from shrubs of various species of Rubiaceous genera, Cinchona and Remijia was the first successful use of a chemical against malaria. QN, a blood schizonticide is also active against the asexual erythrocytic forms of *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*. In addition, QN is gametocytocidal for *P. malariae* and *P. vivax* but, has no direct activity against the gametocytes of *P. falciparum*. Although resistance to QN is rare, cases have been reported. QN salts may be given orally or intravenously (IV), intramuscularly (IM) or rectally (PR) (Barennes et al., 2006). The intravenous administration is the preferred route for severe *falciparum* malaria achieving peak plasma concentrations

in 1–3 h. Thus QN should be diluted and infused slowly. A loading dose (usually 20 mg kg^{-1} body weight) is given over 4 h followed by maintenance infusions (10 mg kg^{-1}, infused over 2 h every 8–12 h) (Warrell et al., 1990). Moderately elevated QN is associated with several features termed cinchonism (auditory symptoms, gastrointestinal disturbances, vasodilation, headache, nausea and blurred vision) (Taylor and White, 2004), hypoglycaemia (Dyson et al., 1985), haemolytic–uraemic syndrome (Gottschall. et al., 1991), and thrombocytopaenia (Glynne et al., 1999). These adverse effects are considered to be the root cause of poor compliance to QN. Clinically, QN toxicity is observed in self-poisoning like the use of excessive QN in the hope of achieving abortion.

4.2. Chloroquine (CQ)

CQ is a rapid acting schizontocide against *P. falciparum* with gametocytocidal activity against asexual erythrocytic forms of *P. malariae*, *P. ovale* and *P. vivax*. CQ remains the most frequently used drug option for *falciparum* malaria in sub-Saharan Africa countries (Winstanley et al., 2004) despite the emergence resistant parasites. Consequently, CQ can no longer be considered an adequately effective therapy of *P. falciparum* in these areas of Africa. Of note, several east African countries have replaced CQ with sulphadoxine–pyrimethamine (SP, Fansidar). To rescue the therapeutic efficacy of CQ, approaches undertaken include the development of amodiaquine following structural modifications of the parent compound and linkage to a transition metal molecule to produce ferroquine (Kreidenweiss et al., 2006).

CQ is slowly eliminated from the body and hence accumulates in certain organs and tissues of the body such as the adrenal glands and alters physiological function of the organs. Studies indicate adverse CQ effects on kidney (Musabayane et al., 1996), cardiovascular system (Siqueira-Batista et al., 1998) and the retina (Ferreras et al., 2007).

4.3. Mefloquine

Mefloquine, a quinoline derivative structurally related to QN is a blood schizontocide which inhibits the asexual stages of *P. falciparum and P. vivax*, but has no effect on the hepatic stage of malaria parasites. Resistance to mefloquine has been reported leading to the use of combinations of mefloquine with sulphadoxine/pyrimethamine (SP) or mefloquine/artemisinin derivatives/combinations. Neuropsychiatric adverse events associated with mefloquine prophylaxis have been reported (Winstanley, 1996). In view of the long half-life of the drug, its administration in areas with intensive malaria transmission is contra-indicated.

4.4. Primaquine (PQ)

The 8-aminoquinoline, PQ is a schizonticide used to eradicate the pre-erythrocytic liver latent tissue forms of *P. vivax* and *P. ovale* which cause malaria relapses. Whilst PQ remains the drug of choice to eradicate and control hypnozoites of *P. vivax* and *P. ovale* P. vivax the antimalarial may precipitate haemolytic anaemia in glucose-6-phosphate dehydrogenase (G-6-PD) deficient patients (Burgoine et al., 2010). Against this background is evidence suggests, however, that a course of 8 weekly doses may be a safe and effective alternative to the traditional 14 day course of the drug (Myat-Phone-Kyaw et al., 1994). In *P. falciparum* infection, a single dose of 0.75 mg/kg is given against the gametocytes (excluding patients <4 years and pregnant women) and is still superior to artemisinin derivatives. This dose can also be tolerated by G6PD deficient patients. PQ also eradicates the primary exoerythrocytic stage of *Plasmodium* species as well as gametocytocidal and sporontocidal phases. PQ is orally administered daily due to its short half life and high doses are

associated with haematological disorders and gastrointestinal disturbances (Baird and Rieckmann, 2003). Transdermal PQ delivery studies may overcome these shortfalls (Mayorga et al., 1998).

5. Anti-folates

The inhibition of *falciparum* folate metabolism remains an attractive target for malaria treatment. Anti-folate derivatives interfere with folate metabolism, a pathway essential for survival of the parasite via inhibition of enzymes dihydrofolate reductase (DDFR) (Bzik et al., 1987) and dihydropteroate synthase (DHPS) (Lu et al., 2010). The combination of pyrimethamine, an inhitor of DHFR, and sulphadoxine (SD) an inhibitor of DHPS, has been widely used for the treatment of malaria, but due to the development of resistance to DHPS and DFHR antagonists the activity has been lost in most parts of the world including tropical Africa.

5.1. Sulphadoxine/pyrimethamine (SP) combination

SP drug combination is the most widely used option for uncomplicated *P. falciparum* malaria in several African countries because it is affordable and practicable. SP combination has a long-life and is, therefore, prone to the rapid emergence of resistance *falciparum* parasites due to the slow elimination from the body (Nwanyanwu et al., 1996; Mugittu et al., 2005). Pyrimethamine is also formulated in fixed combination with sulpalene or dapsone.

5.2. Proguanil

Proguanil, a folate antagonist which destroys the malaria parasite by inhibiting DDFR is used for malaria prophylaxis in some countries (Mulenga et al., 2006). However, clinical failure rates have cast a shadow on the drug's further development.

6. New anti-malarial drugs

Strategies in the discovery and development of new anti-malarial drugs have ranged from minor modifications and reformulation of existing drugs to designing agents that act against new targets. Currently, several treatment options have been developed based on existing anti-malarial drugs in contrast with the period when CQ monotherapy was the standard treatment. This section briefly describes chemotherapeutic agents that have been developed to improve malaria treatment.

6.1. Artemether/lumefantrine combination

Current WHO guidelines recommend the ACT artemether/lumefantrine (AL) for the treatment of uncomplicated malaria caused by *Plasmodium falciparum* (WHO, 2008). Artemether is a methyl-ether derivative of artemisinin with blood schizontocidal and gametocytocidal activities while lumefantrine is a racemic fluorine derivative with high blood schizontocidal activity (Wernsdorfer et al., 1998). Artemether with a half-life of 2–3 h is easily absorbed and rapidly eliminated from plasma, whereas lumefantrine with a half-life of three to six days is eliminated slowly and thus provides a high long-term cure rate. Therefore, the complementary pharmacokinetics and dissimilar modes of action of AL provides synergistic anti-malarial activity and hence rapid clearance of parasitaemia after a short treatment course. Studies have shown AL to be effective both in sub-Saharan Africa and in areas with multi-drug resistant *P. falciparum* in Southeast Asia (Hutagalung et al., 2005; Kokwaro et al., 2007; Achan et al., 2009). It should be noted that AL combination is active against the blood stages of *P. vivax*, but is not active against

hypnozoites. Therefore, an 8-amino-quinoline derivative such as PQ should be given sequentially after the combination in cases of mixed infections of *P. falciparum* and *P. vivax* to achieve hypnozoites eradication. Several studies have confirmed the safety and tolerability of AL in in a wide range of patient populations including children (van Vugt et al., 1999; Falade et al., 2005; Abdulla et al., 2008; Hatz et al., 2008).

6.2. Dihydroartemisinin (DHA) and piperaquine

Several studies indicate that the dihydroartemisinin (DHA) and piperaquine (PQP) combination has the potential to be an effective anti-malarial drug against multi-drug resistant *falciparum* malaria (Giao et al., 2004; Ashley et al., 2004, 2005; Zwang et al., 2009). The DHA/PQP combination rapidly reduces parasite biomass in the patient through the brief yet potent activity of DHA and the subsequent removal of uncleared parasites by the less active but more slowly eliminated PQP (Tarning et al., 2005). DHA, an artemisinin derivative, and PQP, a bisquinoline, have elimination half-lives of approximately 1 h (Newton et al., 2000) and approximately 2–3 weeks (Hung et al., 2003, 2004), respectively. The two components of DHA and PQP provide a combination that is relatively inexpensive and has been shown to be effective both in curing malaria and preventing re-infection (Zwang et al., 2009). DHA/PQP combination is well tolerated by all age groups. A major concern with the DHA/PQP combination is that the long half-life of PQP will facilitate the selection of drug resistant parasites.

7. Optimization of therapy

A combination of new as well as old anti-malarial agents has been used as first-line therapy for malaria in Africa and other areas with widespread *Plasmodium* drug resistance. The options include amodiaquine/sulpadoxine/pyrimethamine (Ogungbamigbe et al., 2008), atreminisin (Kublin et al., 2002), chlorproguanil/dapsone (Fanello et al., 2008) and atovaquone/proguanil (Srivastava and Vaidya, 1999).

7.1. Analogues of existing agents

Quinine analogues (CQ, PQ and mefloquine) were developed through chemical strategies to improve on the chemotherapeutic effect of the anti-malarial drug http://jeb.biologists.org/cgi/content/full/206/21/3735-REF81. Analogues of 4-aminoquinolines closely related to CQ such as PQ, a bisquinoline have also been developed and appear to offer the anti-malarial potency of the parent drug, even against CQ-resistant parasites (Kaschula et al., 2002). Additionally, the toxicity of halofantrine, a drug which has a structure closely related to QN has led to the development of an analogue lumefantrine which is now a component of the new combination co-artemether (artemether/lumefantrine) (van Vugt et al., 2000). The same strategy has been applied leading to the discovery of new artemisinins derivatives (endoperoxides) (Posner et al., 2003) and folate antagonists (Tarnchompoo et al. (2002).

7.2. Amodiaquine (AQ)/sulphadoxine/pyrimethamine (SP)

Amodiaquine (AQ) was widely used in various malaria endemic areas until WHO withdrew its endorsement for malaria control programme in 1990 as a result of reports of rare but severe toxic effects (Olliaro et al., 1996). However, there is renewed interest in AQ as a possible alternative to CQ, as it is effective even in areas of intense CQ resistance and has a side-effect profile similar to that of CQ and SP (Brasseur et al., 1999; Gorissen et al., 2000; Staedke et al.,

2001). Several studies indicate that AQ is effective in treating CQ-resistant *P. falciparum* malaria parasites (Olliaro et al., 1996; Van Dillen et al., 1999), despite the reported hematological side effects (Phillips-Howard and West, 1990). Increasing *P. falciparum* resistance to CQ in sub-Saharan Africa necessitates use of alternative antimalarial agents. One alternative regimen, amodiaquine (AQ) plus SP, has shown surprisingly good efficacy in Uganda (Staedke et al., 2001; Dorsey et al., 2002; Gasasira et al., 2003). The SP/AQ antimalarial combination which has shown surprisingly good efficacy for treatment of uncomplicated malaria offers low-cost option for in Africa.

7.3. Natural products

Extensive evaluation of naturally occurring products and medicinal plants has been reported for therapies of many human diseases. Indeed, natural products are the sources of the two most important drugs (QN and artemisinins derivatives) currently available to treat severe *falciparum* malaria. Literature evidence indicates that research approaches aimed at optimizing malaria chemotherapy therapy include modifications of existing agents with natural products. Such drugs at the pre-clinical stages or currently undergoing clinical trials have been reported in literature, but have not been discussed as they are outside the scope of this review.

8. Novel anti-malarial drug delivery systems

The discussion in the in the preceding sections highlights the possibility of developing of anti-malarial drugs and novel delivery formulations that are envisaged to improve the efficacy, specificity, tolerability and therapeutic index of existing drugs. Such formulations are likely to modify the traditional oral dosing schedule of drugs such as CQ whose toxicity has been attributed to transiently high plasma concentrations following oral administration (Siqueira-Batista et al., 1998). Studies indicate successful controlled release of CQ from the synthetic polymer, Eudragit RS 100 (Ndesendo et al., 1996) as well as from pectin (polygalacturonic acid) (Musabayane et al., 2003). Synthetic polymers are disadvantaged by the use of organic solvents and hence relatively harsh formulation conditions. These novel drug delivery systems are likely to optimise the therapeutic efficacy of anti-malarials. Novel drug delivery systems investigated to date include CQ-pectin formulations (Musabayane et al., 2003) lipid nanoemulsion or liposome-entrapping of PQ (Green et al., 2004). Biodegradable natural polymers (albumin, gelatin, alginate, collagen and chitosan) and synthetic polymers (lactide, glycolide, poly(lactide-co-glycolide) (PLGA) have been used as drug delivery systems although they have a relatively short duration of drug release (Rytting et al., 2008).

8.1. Liposomes

Liposomes are lipid vesicles used extensively for controlled delivery drug formulations. Liposome formulations inhibit rapid clearance by controlling the size, charge, and surface hydration of the drug. Studies show that liposomes are effective carriers of anti-malarial drugs (Singh and Vingkar, 2008) as evidenced by that they increase bioavailability of arteminisin derivatives (Gabriels and Plaizier-Vercammen, 2003) to avert malaria recrudescence in experimental animals (Chimanuka et al., 2002). Furthermore, reports indicate sustained prolonged CQ release from liposome formulations (Owais et al., 1995) and PQ (Dierling and Cui, 2005). In addition, CQ- and PQ formulations targeting erythrocytes (Chandra, 2007) and the liver (Singh and Vingkar, 2008) have been developed. Enhanced safety and efficacy have also been achieved for a wide

Table 2
Transdermal formulations of malaria chemotherapeutic agents.

Drug	Formulation	Main findings	References
Aminoquinolines			
CQ	CQ/pectin hydrogel matrix	Sustained controlled CQ release	Musabayane et al. (2003)
PQ	PQ/- Eudragit® RL 100-, ethyl cellulose polymers with various penetration enhancers (Mygliol® 840, Labrafac Hydrophile®, Transcutol®, propylene glycol, oleic acid, etc.)	Ethyl cellulose-based formulations with Mygliol®840 achieved the high plasma PQ	Mayorga et al. (1996)
	PQ/ethyl cellulose polymer mixed with Mygliol® 840 penetration enhancer plus the antioxidant alpha-tocopherol	Sustained controlled PQ release over 40 h	Mayorga et al. (1997)
	PQ/Starch with Mygliol® 840 as a penetration enhancer	Controlled PQ release; with no enhancing effect.	Jeans and Heard (1999)
	PQ with Mygliol® 840 as a penetration enhancer	Extensive binding of PQ to corneocyte keratin which has a significant effect on reservoir formation and permeability	Heard et al. (2003)
Artemisinin derivatives			
Artelinic acid	Artelinic acid/methyl cellulose with combined penetration enhancers dimethyl phthalate/azone	Prophylaxis and rapid elimination of parasites in experimental animals	Klayman et al. (1991)
Artemisinin, artemether, dehydroartemisin	Artemisinin derivatives/ethyl cellulose with the penetration enhancer dimethyl phthalate.	Prophylactic and curative effects in experimental animals	Lin et al. (1994)
Antibiotics			
Doxycycline	Saturated solutions of doxycycline/Mygliol® 840 and ethanol	Improved doxycycline transport across the skin	Perkins and Heard (1999)
Triclosan	Triclosan/acrylic polymer DuroTak®	Improved triclosan transport across the skin	Chedgzoy et al. (2002)

range of drug classes, including antitumor agents, antivirals, antifungals, antimicrobials, vaccines and gene therapeutics (Sharma and Sharma, 1997).

8.2. Nanoparticles

Polymeric nanoparticle devices are biocompatible, slowly hydrolyzed polymer devices such as poly lactide gelatin, albumin, polyalcrylamide, polyisohexylcyanoacrylate and polydiethylmethylidene malonate that control delivery of drugs. Polymeric nanoparticle devices have been used to deliver anti-malarial drugs, halofantrine (Legrand et al., 2003), *Artemisia annua* derivatives (Wan et al., 1992) and CQ (Agrawal et al., 2007) in experimental animals. An added advantage of nanoparticle delivery devices is that they can passively deliver drugs to lysosomes of phagocytes of the mononuclear phagocyte system, following intravenous administration (Rodrigues et al., 1994). The release of the drug at the site following lysosomal degradation enhances the efficacy of the active agent. Indeed, pharmacokinetic evaluation of the albumin-encapsulated PQ targeted to the mouse liver showed significantly higher concentrations of PQ in liver tissues relative to free drug (Green et al., 2004). Gelatine nanoparticles or galactose-coated drug dendrimers have also been studied for controlled PQ delivery (Agrawal et al., 2007). Reports indicate that PQ loaded-poly(D,L-lactide) nanoparticles were not only tolerated by healthy and *Leishmania donovani*-infected mice, but also reduced the 50% lethal dose when compared to the free PQ (Rodrigues et al., 1994).

8.3. Ceramic implants

Polymers (polylactic acid, gelatin or chitosan) are matrices for ceramic particles that can introduce a tailored biodegradable drug. Gelatine ceramic implants have been reported to sustain consistent therapeutic blood CQ concentrations in experimental animals (Saparia et al., 2001).

8.4. Microemulsions

Microemulsions drug delivery systems containing water, oil and active agent have been used in pharmaceutical and medical practice since the earliest days. Gum arabic (branched polysaccharide) microemulsions have been developed for CQ (Vaziri and Warburton, 1994) and PQ (Nishi and Jayakrishnan, 2004). The drug covalently couples via imine bond to aldehyde groups generated by oxidizing the polysaccharide with periodate and simultaneously

fabricates into microspheres. Other studies indicate sustained controlled CQ release from ethyl cellulose microsphere (Patel et al., 2006). A biodegradable polyphosphoester, poly[[(cholesteryl oxocarbonylamido ethyl) methyl bis(ethylene) ammonium iodide] ethyl phosphate] (PCEP) micelle has also been reported to efficiently deliver CQ *in vitro* (Wen et al., 2004).

8.5. Transdermal delivery

Transdermal delivery provides an alternative route of anti-malarial drugs. The advantages of transdermal route include avoidance of hepatic first-pass metabolism, easy administration and possibility of immediate withdrawal of the treatment. Although, transdermal delivery is limited by low skin permeability, methods such as active cationic liposomal delivery (Nair et al., 2009) and electroporation techniques (Sen et al., 2002) developed in the last decades enhance permeation. Indeed, we reported physiological effects of CQ following topical application of pectin-CQ hydrogel matrix patch on the rat skin using dimethyl sulphoxide as a penetration enhancer (Musabayane et al., 2003). The observations suggest that the anti-malarial can be delivered through the transdermal route. Sustained plasma concentration of PQ with concomitant inhibitory activity on asexual malaria parasites has also been reported in experimental animals following transdermal PQ administration (Mayorga et al., 1997). This is beneficial considering that the prophylactic and therapeutic applications of PQ are hampered by dose dependant adverse effects (Winstanley and Breckenridge, 1987). Table 2 below gives an outline of transdermal delivery studies conducted for anti-malarial drugs.

8.6. Rectal delivery

The rectal drug delivery route has been used to deliver artemisinin formulations (Kuranajeewa et al., 2007) and QN (Barennes et al., 1999) despite the observations that the route is associated with marked inter individual variability and bioavailability of the drugs (Kuranajeewa et al., 2007). The rate and extent of rectal drug absorption are often low possibly due to the relatively small surface area available for drug uptake. The composition of the rectal formulation (solid vs liquid, nature of the suppository base) appears to be an important factor determining the absorption process and pattern of drug release (Yong et al., 2003). Barennes et al. (1999), however, developed a rectally delivered QN cream formulation with improved parasitological efficacy and pharmacokinetics in children with *P. falciparum* malaria relative to intramuscular and

intravenous injections. This has immense public health benefits considering that intramuscular injection of QN has been associated with complications that include injection paralysis (Barennes et al., 1996) and limitations such as being unsuitable for field use or in poorly equipped rural hospitals. Rectal administration is a painless procedure that enables self administration, and reduces the risk of infections from already used needles (Barennes et al., 1996).

8.7. Nasal delivery

The nasal drug delivery route is easily accessible and offers a wide surface area for drug absorption (Illum, 2003). One particular advantage of the nasal route is the simplicity of administration, allowing easy treatment following the first signs of illness. Moreover, the nasal cavity allows the drugs to be delivered directly to the brain via the nasal membrane (Graff and Pollack, 2005) which is vital in cerebral malaria. However, limitations of this route include rapid removal of the drug from the site of deposition by mucociliary clearance by enzymatic degradation, low permeability of the nasal epithelium and erratic bio-availability (Galloway and Chance, 1994). Against this background are observations of effective *Plasmodium* infection treatment in rodents with nasally administered dihydroartemisinin formulations (Touitou et al., 2006).

9. Conclusions and future studies

We have briefly described the ways in which anti-malarial drug regimens are employed and suggested delivery strategies to optimize treatment. Evidence presented in this review indicates that chemotherapy remains one of the main ways of controlling malaria, although the sensitivity of *falciparum* malaria to CQ the main stay therapy drug for malaria has rapidly declined throughout the tropics. This review has briefly highlighted some of the current research in the development of anti-malarial drug formulations. Irrespective of the methods used now, there is no doubt that further research based on fundamental knowledge of existing drugs will yield novel therapeutic strategies and formulation approaches that may improve malaria chemotherapy.

Conflict of interest statement

The authors declare that there is no interest that could be perceived as prejudicing the impartiality of the research reported.

References

Abdulla, S., Sagara, I., Borrmann, S., D'Alessandro, U., González, R., Hame, l.M., Ogutu, B., Mårtensson, A., Lyimo, J., Maiga, H., Sasi, P., Nahum, A., Bassat, Q., Juma, E., Otieno, L., Björkman, A., Beck, H.P., Andriano, K., Cousin, M., Lefèvre, G., Ubben, D., Premji, Z., 2008. Efficacy and safety of artemether/lumefantrine dispersible tablets compared with crushed commercial tablets in African infants and children with uncomplicated malaria: a randomised, single-blind, multicentre trial. Lancet 372, 1819–1827.
Achan, J., Tibenderana, J.K., Kyabayinze, D., Wabwire-Mangen, F., Kamya, M.R., Dorsey, G., D'Alessandro, U., Rosenthal, P.J., Talisuna, A.O., 2009. Effectiveness of quinine versus artemether-lumefantrine for treating uncomplicated falciparum malaria in Ugandan children: randomised trial. BMJ 339, b2763.
Adak, T., Sharma, V.P., Orlov, V.S., 1998. Studies on the *Plasmodium viva* relapse pattern in Dehli, India. Am. J. Med. Hyg. 59 (1), 175–179.
Agrawal, P., Gupta, U., Jain, N.K., 2007. Glycoconjugated peptide dendrimers-based nanoparticulate system for the delivery of chloroquine phosphate. Biomaterials 28 (22), 3349–3359.
Akpalu Jr., A.K., Nyame, P.K., Dodoo, A.N.O., 2005. Amodiaquine-induced dystonic reactions: Case reports and implications for policy change in Ghana. Int. J. Risk Safety Med. 17, 1–2.
Alkadi, H.O., 2007. Antimalarial drug toxicity: A review. Chemotherapy 53 (6), 385–391.
Ashley, E., Krudsood, S., Phaiphun, L., Srivilairit, S., McGready, R., Leowattana, W., Hutagalung, R., Wilairatana, P., Brockman, A., Looareesuwan, L., Nosten, F., White, N.J., 2004. Dose optimisation randomized controlled studies of dihydroartemisinin-piperaquine for the treatment of uncomplicated multi-

drug resistant falciparum malaria in Thailand. J. Infect. Dis. 190, 1773–1782.
Ashley, E.A., McGready, R., Hutagalung, R., Phaiphun, L., Slight, T., Proux, S., Thwai, K.L., Barends, M., Looareesuwan, S., White, N.J., Nosten, F., 2005. A randomized, controlled study of a simple, once-daily regimen of dihydroartemisinin-piperaquine for the treatment of uncomplicated, multidrug-resistant falciparum malaria. Clin Infect Dis. 41, 425–432.
Assefa, A., Kassa, M., Tadese, G., Mohamed, H., Animut, A., Mengesha, T., 2010. Therapeutic efficacy of Artemether/Lumefantrine (Coartem(R) against *Plasmodium falciparum* in Kersa, South West Ethiopia. Parasit. Vectors 3 (1), 1.
Baird, J.K., Rieckmann, K.H., 2003. Can primaquine therapy for vivax malaria be improved? Trends Parasitol. 19 (3), 115–120.
Barennes, H., Balima-Koussoube, T., Nagot, N., Charpentier, J.C., Pussard, E., 2006. Safety and efficacy of rectal compared with intramuscular quinine for the early treatment of moderately severe malaria in children: randomized clinical trial. Br. Med. J. 332 (7549), 1055–1057.
Barennes, H., Pussard, E., Mahaman Sani, A., Clavier, F., Kahiatani, F., Granic, G., Henzel, D., Ravinet, L., Verdier, F., 1996. Efficacy and pharmacokinetics of a new intrarectal quinine formulation in children with *Plasmodium falciparum* malaria. Br. J. Clin. Pharmacol. 41 (5), 389–395.
Barennes, H., Verdier, F., Clavier, F., Pussard, E., 1999. Pharmacokinetics of Quinimax suppositories in children with malaria. Clin. Drug Invest. 17, 287–291.
Bloland, P.B., 2003. A contrarian view of malaria therapy policy in Africa. Am. J. Trop. Med. Hyg. 68 (2), 125–126.
Brasseur, P., Guiguemde, R., Diallo, S., Guiyedi, V., Kombila, M., Ringwald, P., Olliaro, P., 1999. AmodiaquineAmodiaquine remains effective for treating uncomplicated malaria in West and Central Africa. Trans. R. Soc. Trop. Med. Hyg. 93, 645–650.
Burgoine, K.L., Bancone, G., François Nosten, F., 2010. The reality of using primaquine. Malaria Journal 2010 9, 376.
Bzik, D., Li, W., Horii, T., Inselburg, J., 1987. Molecular cloning and sequence analysis of the *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase gene. Proc. Natl. Acad. Sci. U. S. A. 84, 8360–8364.
Chandra, S., 2007. Chloroquine delivery to erythrocytes in *Plasmodium berghei*-infected mice using antibody bearing liposomes as drug vehicles. J. Biosci. 16 (3), 137–144.
Chedgzoy, P., Winckle, G., Heard, C.M., 2002. Triclosan: release from adhesive transdermal formulations and *in vitro* permeation across human epidermal membranes. Int. J. Pharm. 235 (1–2), 229–236.
Chimanuka, B., Gabriëls, M., Detaevernier, M.R., Plaizier-Vercammen, J.A., 2002. Preparation of beta-artemether liposomes, their HPLC-UV evaluation and relevance for clearing recrudescent parasitaemia in *Plasmodium chabaudi* malaria infected mice. J. Pharm. Biomed. Anal. 28 (1), 13–22.
Cox-Singh, J., Davis, T.M., Lee, K.S., Shamsul, S.S., Matusop, A., Ratnam, S., Rahman, H.A., Conway, D.J., Singh, B., 2008. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. Clin. Infect. Dis. 46, 165–171.
Dierling, A.M., Cui, Z., 2005. Targeting primaquine into liver using chylomicron emulsions for potential *vivax* malaria therapy. Int. J. Pharm. 303 (1–2), 148–152.
Dorsey, G., Njama, D., Kamya, M.R., Cattamanchi, A., Kyabayinze, D., Staedke, S.G., Gasasira, A., Rosenthal, P.J., 2002. Sulfadoxine/pyrimethamine alone or with amodiaquine or artesunate for treatment of uncomplicated malaria: a longitudinal randomized trial. Lancet 360, 2031–2038.
Duong, S., Lim, P., Fandeur, T., Tsuyuoka, R., Wongsrichanalai, C., 2004. Importance of protection of anti-malarial combination therapies. Lancet 364 (9447), 1754–1755.
Dyson, E.H., Proudfoot, A.T., Prescott, L.F., Heyworth, R., 1985. Death and blindness due to overdose of quinine. Br. Med. J. 291 (6487), 31–33.
Falade, C., Makanga, M., Premji, Z., Ortmann, C.E., Stockmeyer, M., de Palacios, P.I., 2005. Efficacy and safety of artemether/lumefantrine (Coartem®) tablets (six-dose regimen) in African infants and children with acute, uncomplicated falciparum malaria. Trans. R. Soc. Trop. Med. Hyg. 99, 459–467.
Fanello, C.I., Karema, C., Ngamije, D., Uwimana, A., Ndahindwa, V., Van Overmeir, C., Van Doren, W., Curtis, J., D'Alessandro, U., 2008. A randomised trial to assess the efficacy and safety of chlorproguanil/dapsone plus artesunate for the treatment of uncomplicated *Plasmodium falciparum* malaria. Trans. R. Soc. Trop. Med. Hyg. 102 (5), 412–420.
Ferreras, A., Pinilla, I., Abecia, E., Pajarín, A.B., Honrubia, F.M., 2007. Retinal toxicity following chloroquine therapy. Arch. Soc. Esp. Oftalmol. 82 (2), 103–108.
Gabriels, M., Plaizier-Vercammen, J., 2003. Physical and chemical evaluation of liposomes, containing artesunate. J. Pharm. Biomed. Anal. 31 (4), 655–667.
Galloway, J.A., Chance, R.E., 1994. Improving insulin therapy: achievements and challenges. Horm. Metab. Res. 26 (12), 591–598.
Garnham, P.C.C., Bray, R.S., Bruce-Chwatt, L.J., Draper, C.C., Killick-Kendrick, R., Sergiev, P.G., Tiburskaja, N.A., Shute, P.G., Maryon, M., 1975. A strain of Plasmodium vivax characterized by prolonged incubation: morphological and biological characteristics. Bull. World Health Org. 52 (1), 21–32.
Gasasira, A.F., Dorsey, G., Nzarubara, B., Staedke, S.G., Nassali, A., Rosenthal, P.J., Kamya, M.R., 2003. Comparative efficacy of aminoquinoline-antifolate combinations for the treatment of uncomplicated falciparum malaria in Kampala, Uganda. Am. J. Trop. Med. Hyg. 68, 127–132.
Giao, P.T., de Vries, P.J., Hung, L.Q., Binh, T.Q., Nam, N.V., Kager, P.A., 2004. CV8, a new combination of dihydroartemisinin, piperaquine, trimethoprim and primaquine, compared with atovaquone–proguanil against falciparum malaria in Vietnam. Trop. J. Med. Int. Health 9, 209–216.

Glynne, P., Salama, A., Chaudhry, A., Swirsky, D., Lightstone, L., 1999. Quinine-induced immune thrombocytopenic purpura followed by hemolytic uremic syndrome. Am. J. Kid. Dis. 33, 133–137.

Gorissen, E., Ashruf, G., Lamboo, M., Bennebroek, J., Gikunda, S., Mbaruku, G., Kager, P.A., 2000. In vivo efficacy study of amodiaquine and sulfadoxine/pyrimethamine in Kibwezi, Kenya and Kigoma, Tanzania. Trop. Med. Int. Health 5, 459–463.

Gottschall, J., Elliot, W., Lianos, E., McFarland, J.G., Wolfmeyer, K., Aster, R.H., 1991. Quinine-induced immune thrombocytopenia associated with hemolytic uremic syndrome –a new clinical entity. Blood 77 (2), 306–310.

Graff, C.L., Pollack, G.M., 2005. Functional evidence for p-glycoprotein at the nose-brain barrier. Pharm. Res. 22 (1), 86–93.

Green, M.D., D'Souza, M.J., Holbrook, J.M., Wirtz, R.A., 2004. In vitro and in vivo evaluation of albumin-encapsulated primaquine diphosphate prepared by nebulization into heated oil. J. Microencapsul. 21 (4), 433–444.

Hatz, C., Soto, J., Nothdurft, H.D., Zoller, T., Weitzel, T., Loutan, L., Bricaire, F., Gay, F., Burchard, G.D., Andriano, K., Lefèvre, G., De Palacios, P.I., Genton, B., 2008. Treatment of acute uncomplicated falciparum malaria with artemether/lumefantrine in non-immune populations: a safety, efficacy and pharmacokinetic study. Am. J. Trop. Med. Hyg. 78, 241–247.

Heard, C.M., Monk, B.V., Modley, A.J., 2003. Binding of primaquine to epidermal membranes and keratin. Int. J. Pharm. 257 (1–2), 237–244.

Hung, T.Y., Davis, T.M., Ilett, K.F., Karunajeewa, H., Hewitt, S., Denis, M.B., Lim, C., Socheat, D., 2004. Population pharmacokinetics of piperaquine in adults and children with uncomplicated falciparum or vivax malaria. Br. J. Clin. Pharmacol. 57, 253–262.

Hung, T.Y., Davis, T.M., Ilett, K.F., 2003. Measurement of piperaquine in plasma by liquid chromatography with ultraviolet absorbance detection. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 791, 93–101.

Hutagalung, R., Paiphun, L., Ashley, E.A., McGready, R., Brockman, A., Thwai, K.L., Singhasivanon, P., Jelinek, T., White, N.J., Nosten, F.H., 2005. A randomized trial of artemether-lumefantrine versus mefloquine-artesunate for the treatment of uncomplicated multi-drug resistant Plasmodium falciparum on the western border of Thailand. Malaria J. 22 (4), 46.

Illum, L., 2003. Nasal drug delivery-possibilities, problems and solutions. J. Control. Release 87 (1–3), 187–198.

Jambou, R., Legrand, E., Niang, M., Khim, N., Lim, P., Volney, B., Ekala, M.T., Bouchier, C., Esterre, P., Fandeur, T., Mercereau-Puijalon, O., 2005. Resistance of Plasmodium falciparum field isolates to in-vitro artemether and point mutations of the SERCA-type PfATPase6. Lancet 366 (9501), 1960–1963.

Jeans, C.W., Heard, C.M., 1999. A therapeutic dose of primaquine can be delivered across excised human skin from simple transdermal patches. Int. J. Pharm. 189 (1), 1–6.

Kaschula, C.H., Egan, T.J., Hunter, R., Basilico, N., Parapini, S., Taramelli, D., Pasini, E., Monti, D., 2002. Structure–activity relationships in 4 aminoquinoline antiplasmodials. The role of the group at the 7-position. J. Med. Chem. 45 (16), 3531–3539.

Klayman, D.L., Ager, A.L., Fleckenstein, L., Lin, A.J., 1991. Transdermal artelinic acid: an effective treatment for Plasmodium berghei-infected mice. Am. J. Trop. Med. 45, 602–607.

Kokwaro, G., Mwai, L., Nzila, A., 2007. Artemether/lumefantrine in the treatment of uncomplicated falciparum malaria. Exp. Opin. Pharmacother. 8 (1), 75–94.

Kongpatanakul, S., Chatsiricharoenkul, S., Khuhapinant, A., Atipas, S., Kaewkungwal, J., 2009. Comparative study of dihydroartemisinin and artesunate safety in healthy Thai volunteers. Int. J. Clin. Pharm. Ther. 47 (9), 579–586.

Kreidenweiss, A., Kremsner, P.G., Dietz, K., Mordmüller, B., 2006. In vitro activity of ferroquine (sar97193) is independent of chloroquine resistance in Plasmodium falciparum. Am. J. Trop. Med. Hyg. 75 (6), 1178–1181.

Kublin, J.G., Dzinjalamala, F.K., Kamwendo, D.D., Malkin, E.M., Cortese, J.F., Martino, L.M., Mukadam, R.A., Rogerson, S.J., Lescano, A.G., Molyneux, M.E., Winstanley, P.A., Chimpeni, P., Taylor, T.E., Plowe, C.V., 2002. Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of Plasmodium falciparum malaria. J. Infect. Dis. 185 (3), 380–388.

Kuranajeewa, H.A., Manning, L., Mueller, I., Ilett, K.F., Davis, T.M., 2007. Rectal administration of artemisinin derivatives for the treatment of malaria. JAMA 297 (21), 2381–2390.

Legrand, P., Mosqueira, V., Loiseau, P., Bories, C., Barratt, G., 2003. Long circulating nanocapsules: interest in the treatment of severe malaria with halofantrine. Ann. Pharm. Fr. 61 (3), 196–202.

Lin, A.J., Ager, A.L., Klayman, A.L., 1994. Antimalarial activity of dihydroartemisinin derivatives by transdermal application. Am. Soc. Trop. Med. Hyg. 50 (6), 777–783.

Lu, F., Lim, C.S., Deok Hwa Nam, D.H., Kim, K., Lin, K., Kim, T.S., Lee, H.W., Chen, J.H., Wang, Y., Sattabongkot, J., Han, E.T., 2010. Mutations in the antifolate-resistance-associated genes dihydrofolate reductase and dihydropteroate synthase in Plasmodium vivax isolates from malaria-endemic countries. Am. J. Trop. Med. Hyg. 83 (3), 474–479.

Mayorga, P., Deharo, E., Puisieux, F., Couarraze, G., 1997. Interpretation and prediction of plasma levels of primaquine following transdermal delivery in Swiss mice. Int. J. Pharm. 155 (1), 99–107.

Mayorga, P., Miltgen, F., Coquelin, F., Couarraze, G., 1998. Rodent malaria prophylaxis by transdermal delivery of primaquine. Int. J. Parasitol. 28 (8), 1283–1285.

Mayorga, P., Puisieux, F., Couarraze, G., 1996. Formulation study of a transdermal delivery of primaquine. Int. J. Pharm. 132 (1–2), 71–79.

Mugittu, K., Abdulla, S., Falk, N., Masanja, H., Felger, I., 2005. Efficacy of sulfadoxine-pyrimethamine in Tanzania after two years as first-line drug for uncomplicated malaria: assessment protocol and implication for treatment policy strategies. Malaria J. 4 (1), 55.

Mulenga, M., Malunga, F., Bennett, S., Thuma, P.E., Shulman, C., Fielding, K., Alloueche, A., Greenwood, B.M., 2006. A randomised, double-blind, placebo-controlled trial of atovaquone–proguanil vs. sulphadoxine–pyrimethamine in the treatment of malarial anaemia in Zambian children. Trop. Med. Int. Health 11 (11), 1643–1652.

Musabayane, C.T., Munjeri, O., Matavire, T.P., 2003. Transdermal delivery of chloroquine by amidated pectin hydrogel matrix patch in the rat. Renal Failure 25 (4), 525–534.

Musabayane, C.T., Windle, R.J., Forsling, M.L., Balment, R.J., 1996. Arginine vasopressin mediates the chloroquine induced increase in renal sodium excretion. Trop. Med. Int. Health 1 (4), 542–550.

Myat-Phone-Kyaw, Myint-Oo, Aung-Naing, Aye-Lwin-Htwe, 1994. The use of primaquine in malaria infected patients with red cell glucose-6-phosphate dehydrogenase (G6PD) deficiency in Myanmar. Southeast Asian J. Trop. Med. Public Health 25 (4), 710–713.

Nair, A., Reddy, C., Jacob, S., 2009. Delivery of a classical antihypertensive agent through the skin by chemical enhancers and iontophoresis. Skin Res. Technol. 15 (2), 187–194.

Ndesendo, V.M., Meixner, W., Korsatko, W., Korsatko-Wabnegg, B., 1996. Microencapsulation of chloroquine diphosphate by Eudragit RS100. J. Microencapsul. 13 (1), 1–8.

Newton, P., Suputtamongkol, Y., Teja-Isavadharm, P., Pukrittayakamee, S., Navaratnam, V., Bates, I., White, N., 2000. Antimalarial bioavailability and disposition of artesunate in acute falciparum malaria. Antimicrob. Agents Chemother. 44, 972–977.

Nishi, K.K., Jayakrishnan, A., 2004. A Preparation and in vitro evaluation of primaquine-conjugated gum arabic microspheres. Biomacromolecules 5 (4), 1489–1495.

Noedl, H., Se, Y., Schaecher, K., Smith, B.L., Socheat, D., Fukuda, M.M., 2008. Evidence of artemisinin-resistant malaria in western Cambodia; Artemisinin Resistance in Cambodia 1 (ARC1) Study Consortium. N. Engl. J. Med. 359 (24), 2619–2620.

Nosten, F., White, N.J., 2007. Artemisinin-based combination treatment of falciparum malaria. Am. J. Trop. Med. Hyg. 77 (6), 181–192.

Nwanyanwu, O.C., Ziba, C., Kazembe, P., Chitsulo, L., Willima, J.J., Kumwenda, N., Redd, S.C., 1996. Efficacy of sulphadoxine/pyrimethamine for Plasmodium falciparum malaria in Malawian children under five years of age. Trop. Med. Int. Health 1, 231–235.

Ogbonna, A., Uneke, C., 2008. Artemisinin-based combination therapy for uncomplicated malaria in sub-Saharan Africa: the efficacy, safety, resistance and policy implementation since Abuja 2000. Trans. R. Soc. Trop. Med. Hyg. 102 (7), 621–627.

Ogungbamigbe, T.O., Ojurongbe, O., Ogunro, P.S., Okanlawon, B.M., Kolawole, S.O., 2008. Nigeria: efficacy of amodiaquine plus sulfadoxine-pyrimethamine and chloroquine plus chlorpheniramine for treatment. Mem. Inst. Oswaldo Cruz. 103 (1), 79–84.

Olliaro, P., Nevill, C., LeBras, J., Ringwald, P., Mussano, P., Garner, P., Brasseur, P., 1996. Systematic review of amodiaquine treatment in uncomplicated malaria. Lancet 348, 1196–1201.

Olliaro, P.L., Bloland, P.B., 2001. Clinical and public health implications of antimalarial drug resistance. In: Rosenthal, P. (Ed.), Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery. Humana, Totowa, NJ, pp. 65–83.

Owais, M., Varshney, G.C., Choudhury, A., Chandra, S., Gupta, C.M., 1995. Chloroquine encapsulated in malaria-infected erythrocyte-specific antibody-bearing liposomes effectively controls chloroquine-resistant Plasmodium berghei infections in mice. Antimicrob. Agents Chemother. 39 (1), 180–184.

Patel, V.A., Murthy, R.S.R., Patel, H.V., 2006. Formulation and in vitro characterization of ethyl cellulose microspheres containing chloroquine phosphate. J. Indian Pharm. 5 (51), 82–86.

Perkins, N.C., Heard, C.M., 1999. In vitro dermal and transdermal delivery of doxycycline from ethanol:migliol 840 vehicles. Int. J. Pharm. 190 (2), 155–164.

Petras, J., Young, G.D., Bauman, R.A., Kyle, D.E., Gettayacamin, M., Webster, H., 2000. Arteether-induced brain injury in Macaca mulatta. I. The precerebellar nuclei: the lateral reticular nuclei, the paramedian reticula nuclei, and perihypoglossal nuclei. Anat. Embryol. 20 (5), 383–397.

Phillips-Howard, P.A., West, L.J., 1990. Serious adverse drug reactions to pyrimethamine-sulphadoxine, pyrimethamine-dapsone and to amodiaquine in Britain. J. R. Soc. Med. 83, 82–85.

Posner, G.H., Paik, I.H., Sur, S., McRiner, A.J., Borstnik, K., Xie, S., Shapiro, T.A., 2003. Orally active, antimalarial, anticancer, artemisinin-derived trioxane dimers with high stability and efficacy. J. Med. Chem. 46 (6), 1060–1065.

Rodrigues, J.M., Croft, S.L., Fessi, H., Bories, C., Devissaguet, J.P., 1994. The activity and ultrastructural localization of primaquine-loaded poly (d,l-lactide) nanoparticles in Leishmania donovani infected mice. Trop. Med. Parasitol. 45 (3), 223–228.

Rytting, E., Nguyen, J., Wang, X., Kissel, T., 2008. Biodegradable polymeric nanocarriers for pulmonary drug delivery. Exp. Opin. Drug Deliv. 5 (6), 629–639.

Saparia, B., Solanki, A., Murthy, R.S.R., 2001. Sustained release implants of chloroquine phosphate for possible use in chemoprophylaxis of malaria. Indian J. Exp. Biol. 39 (9), 902–905.

Saussine, A., Loriot, M.A., Picard, C., Lecerf, V., Landry, J., Scheer, I., Voicu, S., Leenhardt, A., Ducrocq, G., Maubec, E., Crickx, B., 2009. Chloroquine cardiotoxicity in long term lupus therapy in two patients. Ann. Dermatol. Venereol. 136 (6–7), 530–535.

Sen, A., Daly, M.E., Hui, S.W., 2002. Transdermal insulin delivery using lipid enhanced electroporation. Biochim. Biophys. Acta 1564 (1), 5–8.

Shanks, G.D., Kain, K.C., Keystone, J.S., 2001. Malaria chemoprophylaxis in the age of drug resistance. II: Drugs that may be available in the future. Clin. Infect. Dis. 33 (3), 381–385.

Sharma, A., Sharma, U., 1997. Liposomes in drug delivery: progress and limitations. Int. J. Pharm. 154, 123–140.

Shekalaghe, S.A., ter Braak, R., Daou, M., Kavishe, R., van den Bijllaardt, W., van den Bosch, S., Koenderink, Jan B., Luty, A.J.F., Whitty, C.J.M., Drakeley, C., Sauerwein, R.W., Bousema, T., 2010. In Tanzania, hemolysis after a single dose of primaquine coadministered with an artemisinin is not restricted to glucose-6-phosphate dehydrogenase-deficient (G6PD A-) individuals. Antimicrob. Agents Chemother. 54 (5), 1762–1768.

Singh, K.K., Vingkar, S.K., 2008. Formulation, anti-malarial activity and biodistribution of oral lipid nanoemulsion of primaquine. Int. J. Pharm. 347 (1–2), 136–143.

Siqueira-Batista, R., Ramos Junior, A.N., Pessanha, B.S., Sforza-de Almeida, M.P., Potsch, D.F., 1998. Chloroquine and cardiac arrhythmia: case report. East Afr. Med. J. 75 (2), 117–119.

Sirsat, R.A., Dasgupta, A., 1997. Haematological complications of proguanil in a patient with chronic renal failure. Nephron 97 (1), 108.

Srivastava, I.K., Vaidya, A.B., 1999. A mechanism for the synergistic antimalarial action of atovaquone and proguanil. Antimicrob. Agents Chemother. 43 (6), 1334–1339.

Staedke, S.G., Kamya, M.R., Dorsey, G., Gasasira, A., Ndeezi, G., Charlebois, E.D., Rosenthal, P.J., 2001. Amodiaquine, sulfadoxine/pyrimethamine, and combination therapy for treatment of uncomplicated falciparum malaria in Kampala, Uganda: a randomized trial. Lancet 358, 368–374.

Tafazoli, S., O'Brien, P.J., 2009. Amodiaquine-induced oxidative stress in a hepatocyte inflammation model. Toxicology 256 (1–2), 101–109.

Tarnchompoo, B., Sirichaiwat, C., Phupong, W., Intaraudom, C., Sirawaraporn, W., Kamchonwongpaisan, S., Vanichtanankul, J., Thebtaranonth, Y., Yuthavong, Y., 2002. Development of 2,4- diaminopyrimidines as antimalarials based on inhibition of the S108N and C59R+S108N mutants of dihydrofolate reductase from pyrimethamine resistant *Plasmodium falciparum*. J. Med. Chem. 45 (6), 1244–1252.

Tarning, J., Lindegardh, N., Annerberg, A., Singtoroj, T., Day, N.P., Ashton, M., White, N.J., 2005. Pitfalls in estimating piperaquine elimination. Antimicrob. Agents Chemother. 49, 5127–5128.

Taylor, W.R.J., White, N.J., 2004. Antimalarial drug toxicity: a review. Drug Safety 27 (1), 25–61.

Touitou, E., Waknine, J.H., Godin, B., Golenser, J., 2006. Treatment of malaria in a mouse model by intranasal drug adminstration. Int. J. Parasitol. 6 (14), 1493–1498.

Van Dillen, J., Custers, M., Wensink, A., Wouters, B., van Voorthuizen, T., Voorn, W., Khan, B., Muller, L., Nevill, C., 1999. A comparison of amodiaquine and sulfadoxine-pyrimethamine as firstline treatment of falciparum malaria in Kenya. Trans. R. Soc. Trop. Med. Hyg. 93, 185–188.

van Vugt, M., Looareesuwan, S., Wilairatana, P., McGready, R., Villegas, L., Gathmann, I., Mull, R., Brockman, A., White, N.J., Nosten, F., 2000. Artemether-lumefantrine for the treatment of multidrug-resistant falciparum malaria. Trans. R. Soc. Trop. Med. Hyg. 94 (5), 545–548.

van Vugt, M.V., Wilairatana, P., Gemperli, B., Gathmann, I., Phaipun, L., Brockman, A., Luxemburger, C., White, N.J., Nosten, F., Looareesuwan, S., 1999. Efficacy of six doses of artemether-lumefantrine (benflumetol) in multidrug-resistant *Plasmodium falciparum* malaria. Am. J. Trop. Med. Hyg. 60, 936–942.

Vaziri, A., Warburton, B., 1994. Slow release of chloroquine phosphate from multiple taste-masked W/O/W multiple emulsions. J. Microencapsul. 11 (6), 641–648.

Vijaykadga, S., Rojanawatsirivej, C., Cholpol, S., Phoungmanee, D., Nakavej, A., Wongsrichanalai, C., 2006. *In vivo sensitivity monitoring of mefloquine monotherapy and artesunate-mefloquine combinations for the treatment of uncomplicated falciparum malaria in Thailand in 2003*. Trop. Med. Int. Health 11, 211–219.

Wan, Y.D., Zang, Q.Z., Wang, J.S., 1992. Studies on the antimalarial action of gelatin capsule of Artemisia annua. Zhonqquo ji shenq chonq xue yu ji shenq chonq bing za zhi 10 (4), 290–294.

Warrell, D.A., Molyneux, M.E., Beales, P.F., 1990. Severe and complicated malaria. Trans. R. Soc. Trop. Med. Hyg. 84, S1–S65.

Watkins, W.M., Masobo, M., 1993. Treatment of *Plasmodium falciparum* malaria with pyrimethamine-sulfadoxine: selective pressure for resistance is a function of long elimination half-life. Trans. R. Soc. Trop. Med. Hyg. 87 (1), 75–78.

Wen, J., Mao, H., Li, W., Lin, K.Y., Leong, K.W., 2004. Biodegradable polyphosphoester micelles for gene delivery. J. Pharm. Sci. 93 (8), 2142–2157.

Wernsdorfer, W.H., Landgraf, B., Kilimali, V.A., Wernsdorfer, G., 1998. Activity of benflumetol and its enantiomers in fresh isolates of *Plasmodium falciparum* from East Africa. Acta Trop. 70, 9–15.

Winstanley, P.A., Breckenridge, A.M., 1987. Currently important antimalarial drugs. Ann. Trop. Med. Parasitol. 81 (5), 619–627.

Winstanley, P.A., Ward, S., Snow, R., Breckenridge, A., 2004. Therapy Falciparum Malaria in Sub-Saharan Africa: from Molecule to policy. Clin. Microbiol. Rev. 17 (3), 612–637.

Winstanley, P.A., 1996. Mefloquine: the benefits outweigh the risks Brit. J. Clin. Pharmacol. 42 (4), 411–413.

World Health Organization, 2006. Guidelines for the treatment of malaria (1st ed.). Report no WHO/HTM/MAL/2006.1108. World Health Organization, Geneva.

World Health Organization, 2008. World malaria report WHO/HTM/GMP/2008.1. WHO, Geneva.

Yong, C.S., Sah, H., Jahng, Y., Chang, H.W., Son, J.K., Lee, S.H., Jeong, T.C., Rhee, J.D., Baek, S.H., Kim, C.K., Choi, H.G., 2003. Physicochemical characterization of diclofenac sodium-loaded poloxamer gel as a rectal delivery system with fast absorption. Drug Dev. Ind. Pharm. 29 (5), 545–553.

Zwang, J., Ashley, E.A., Karema, C., D'Alessandro, U., Smithuis, F., 2009. Safety and efficacy of dihydroartemisinin-piperaquine in falciparum malaria: a prospective multi-centre individual patient data analysis. PLoS ONE 4 (7), e6358.