UNIVERSITY OF KWAZULU-NATAL

EVALUATION OF THE EFFICACY OF TRANSDERMAL DELIVERY OF CHLOROQUINE ON MALARIA PARASITES IN *PLASMODIUM BERGHEI*-INFECTED MALE SPRAGUE-DAWLEY RATS: EFFECTS ON BLOOD GLUCOSE AND RENAL ELECTROLYTE HANDLING

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EVALUATION THE OF THE EFFICACY OF TRANSDERMAL DELIVERY OF CHLOROQUINE ON MALARIA PARASITES IN *PLASMODIUM BERGHEI*-INFECTED MALE SPRAGUE-DAWLEY RATS: EFFECTS ON BLOOD GLUCOSE AND RENAL ELECTROLYTE HANDLING

By

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Submitted in fulfilment of the requirements for the degree of Master of Medical Science in Human Physiology in the Discipline of Human Physiology, School of Laboratory Medicine and Medical Sciences, Faculty of Health Sciences

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DECLARATION

I, **Happiness Sibiya**, hereby declare that the dissertation entitled **"Evaluation of the efficacy of transdermal delivery of chloroquine on malaria parasites in *Plasmodium berghei*-infected male Sprague-Dawley rats: effects on blood glucose and renal electrolyte handling"** is a result of my own investigation and research and that this work 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, the work used is duly acknowledged in the text.

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LIST OF ABBREVIATIONS

α	Alpha
ANOVA	One analysis of variance
ACT	Artemisinin combination therapy
ATP	Adenosine triphosphate
AVP	Arginine vasopressin
β	Beta
BRU	Biomedical Resources Unit
b.wt	Body weight
Ca^{+}	Calcium
Cl^{-}	Chloride
CHQ	Chloroquine
cAMP	Cyclic adenosine monophosphate
DA	Degree of amidation
DM	Degree of methylation
DMSO	Dimethyl sulphoxide
ELISA	Enzyme-linked immunosorbant assay
GFR	Glomerular filtration rate
h	Hour
H_2SO_4	Sulphuric acid
IFN-γ	Interferon gamma
IL-10	Interleukin 10
K^{+}	Potassium

LTD	Limited
l	Litre
Kg	Kilogram
MAP	Mean arterial pressure
µ	Micro
µg	Microgram
µl	Microlitre
mg	Milligram
mmHg	Millimetres of Mercury
mmol	Millimole
NO	Nitric oxide
NIC	Non-infected control
IC	Infected control
pRBC	Parasitized red blood cells
pmol	Picomole
p.o	per os (Orally)
RA	Rheumatoid arthritis
RBC	Red blood cell
SC	Stratum corneum
SEM	Standard error of means
SLE	Systemic erythematosus
SP	Sulphadoxine/pyrimethamine
TDD	Transdermal drug delivery

TDDs	Transdermal drug delivery systems
TNF-α	Tumor necrosis factor alpha
UK	United Kingdom
UKZN	University of KwaZulu-Natal
USA	United States of America
WHO	World health organization

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ABSTRACT

Malaria remains a leading cause of morbidity and mortality in tropical regions of the world despite the numerous global efforts to control and manage the disease through prevention and drug intervention. An estimated 300–500 million people are affected by malaria each year, resulting in 1.5–2.7 million mortalities annually. Recent reports indicate that 90% of malaria-related deaths occur in the Sub-Saharan Africa, where a child under five years of age dies every 30 seconds. The development of immunity and increasing resistance to affordable antimalarial drugs is believed to be one of the factors that have led to the increasing number of malaria cases. As a result, the WHO has recommended the use of artemisinin combination therapies (ACTs) for management of malaria. However, these have proven to be very costly and inaccessible in many low-income countries. Therefore, CHQ has remained the mainstay therapy for malaria prophylaxis and treatment despite development of *Plasmodium falciparum* resistance to this drug. The decrease in CHQ efficacy is attributed to a number of factors which are associated with the conventional oral route of administration. CHQ has a bitter, unpalatable taste when administered orally which, more often than not lead to patient's non-compliance. This is thought to be one of the factors that have led to the increasing number of CHQ resistant strains of *P. falciparum*. Moreover, when administered orally, CHQ is reported to deposit in epithelial cells of many vital organs including the liver, heart and kidneys where the drug elicits adverse effects on physiological functions. Hypoglycaemia and impaired renal fluid and electrolyte handling are some of the adverse effects associated with oral administration of CHQ. The present study was designed to develop a novel CHQ-formulation that delivers sustained slow CHQ release into the systemic circulation. We also investigated the ability of this CHQ formulation to clear the malaria parasites in *Plasmodium berghei*-infected male Sprague-Dawley rats. The other objective of the study was to investigate and distinguish between the patho-physiological effects of malaria parasites and CHQ treatment on blood glucose homeostasis and renal fluid and electrolyte handling in rats.

The studies were carried out over a period of 3 weeks, divided into pre-treatment (days 0-7), treatment (days 8-12) and post treatment (days 13-21) periods. To distinguish between the effects of CHQ treatment and malaria infection on blood glucose homeostasis and renal function, separate groups of non-infected and *P. berghei*-infected male Sprague Dawley rats (90g-150g) were used. The animals were housed individually in Makrolon polycarbonate metabolic cages. During the 5-day treatment period, the animal were treated with either oral

CHQ (30 mg), twice daily, 8 hours apart or a once off topical application of the pectin-CHQ matrix patch (56 mg). To evaluate the ability of our novel CHQ-formulation to clear the malaria parasites, pectin-CHQ matrix patches containing 28 and 56 mg of CHQ were used. These doses were calculated based on current clinical doses for the treatment of malaria. However, to assess the short-term effects of our CHQ formulation, a pectin-CHQ patch containing 56 mg CHQ was used. This dose was chosen based on preliminary studies. Percentage parasitaemia, mean body weight changes, food and water intake, mean arterial pressure (MAP), blood glucose concentration, , haematocrit, and 24 hour urine volume voided, Na^+, K^+ and Cl^- were monitored every third day during the pre-treatment and post-treatment periods. However, during the treatment period, all these parameters were monitored daily. To assess the effects of CHQ treatments on some biochemical parameters such as plasma CHQ profiles, electrolytes, AVP and insulin concentrations, separate groups of animals (n=6) were sacrificed during pre-treatment at days 0 and 7, treatment period at days 8, 9, 11, 12 and post-treatment period at day 21.

The results indicate that we were able develop a novel CHQ-formulation and achieved drug percentage incorporations ranging between 52-74%. Furthermore, the amounts of CHQ obtained in the patch compare to clinical doses. We have also demonstrated the ability of the pectin-CHQ matrix patch formulation to deliver sustained controlled therapeutic doses of CHQ which cleared the malaria parasites within a period of 5 days. Oral administration of CHQ significantly decreased blood glucose concentrations of non-infected and *P. berghei* infected rats with a concomitant increase in plasma insulin concentration. Animals treated with a once off- topical application of the pectin-CHQ matrix patch presented with blood glucose concentrations which were comparable to those of untreated non-infected control animals. Both malaria infection and oral CHQ treatment significantly reduced food intake, water intake and % body weight changes by comparison with untreated non-infected control animals. In addition, oral CHQ was associated with increased urinary Na^+ and K^+ outputs, which were mediated through increased plasma AVP concentrations and red blood cells haemolysis respectively. The *P. berghei* parasites and oral CHQ treatment demonstrated blood pressure lowering effects without any change in GFR. On the other hand the once off transdermal application of the pectin-CHQ matrix patch had no significant effects on 24 hour urinary Na^+, K^+ outputs and MAP.

The current study has demonstrated that the pectin-CHQ matrix patch formulation not only clears the malaria parasites from the systemic circulation, but sustains normal blood glucose concentrations and does not affect renal function of malaria infected animals. This suggests that transdermal CHQ delivery has the potential to ameliorate the pathophysiological effects that are associated with oral CHQ treatment and could provide an alternative method for the management of malaria.

CHAPTER 1

INTRODUCTION

1.1.Background

Malaria is a large cause of morbidity and mortality worldwide (Tuteja, 2007). According to the World Health Organization (WHO), malaria is responsible for approximately 660 000 deaths, with Sub-Saharan Africa being the most affected (WHO, 2013). Recent reports indicate that 90% of malaria-related deaths occur in Sub-Saharan Africa (WHO, 2013), where a child under five years of age dies every 30 seconds (WHO, 2000). This infectious disease remains a major health and socioeconomic problem in many low-income countries. In recent years, the burden of the disease and mortality has increased significantly and transmission has spread to new areas (le Sueur *et al.*, 1996). The major cause of this increased mortality and transmission rates is the development of resistance to affordable chemotherapies (Greenwood & Mutabingwa, 2002) and insecticides (Hemingway *et al.*, 2002). Novel approaches that might improve the efficacy of the already existing antimalarial drugs are needed.

Malaria is caused by protozoan parasites of the *Plasmodium* genus. There are four species which cause human malaria, but *Plasmodium falciparum* (*P. falciparum*) causes nearly all the deaths and malaria associated complications (Tuteja, 2007). Chloroquine, a 4-aminoquinoline has and is still used widely for both treatment and prophylaxis of malaria. Due to the increasing number of CHQ resistant *P. falciparum* strains, the WHO has recommended the use of artemisinin combination therapies (ACTs) such as artesunate-amodiaquine, artemether-lumifantrine and artesunate-mefloquine. However, these have proven to be very costly and inaccessible to many developing countries. Hence, CHQ remains the primary drug for the treatment and prophylaxis in some malaria endemic areas, including Sub-Saharan Africa. Chloroquine has been reported to elicit adverse effects on blood glucose homeostasis following oral administration (White *et al.*, 1983). In addition, current research has shown that oral CHQ also evokes adverse effects on renal electrolyte handling (Musabayane *et al.*, 1993). CHQ has been reported to cause cardiac nerve impulse conduction disturbances (Sanguinetti & Jurkiewicz, 1990, Shah, 2005, Owens & Nolin, 2006) and hypotension (Looareesuwan *et al.*, 1986). All these adverse complications are believed to be partly attributed to transiently high plasma CHQ concentrations following oral administration

and/or malaria parasites. Some studies have recorded peak plasma CHQ concentrations ranging between 65 and 263 ng/ml following single oral dose of 10 mg/kg (Walker *et al.*, 1983). CHQ has a long half-life (1-3 months) and a slow elimination rate (Gustafsson *et al.*, 1983). The drug is deposited in tissues such as the heart, kidney and liver, where is disrupts physiological functions such as glucose homeostasis and renal functions. Hence, there is a need for the search of alternative CHQ delivery systems. These systems must try and avoid the use of high drug concentrations, and therefore evade the accumulation of CHQ in tissues. Transdermal drug delivery has been reported to provide a sustained controlled release of drugs directly into the systemic circulation (Prausnitz & Langer, 2008). In addition, we have observed that transdermal administration of CHQ via the pectin CHQ-matrix patch formulation sustains controlled release of CHQ into the bloodstream in non-infected experimental animals (Musabayane et al., 2003). Hence the present study investigated the efficacy of transdermal delivery of CHQ via the pectin CHQ-matrix patch on *Plasmodium berghei* infected rats. We also evaluated the short-term effects of this novel CHQ formulation on blood glucose concentration and renal electrolyte handling in malaria infected rats.

1.2. The *Plasmodium* life cycle

The *Plasmodium* species exhibits a complex life cycle requiring a vector and a vertebrate host (Figure 1). The infection is initiated when sporozoites are injected into the host's bloodstream by a feeding female *Anopheles* mosquito (Tuteja, 2007). These sporozoites quickly migrate to the liver where they invade hepatic cells. Within the hepatic cells, the sporozoites undergo asexual reproduction referred to as exoerythrocytic schizogony. This asexual stage results in the production of merozoites, which are then released into the circulatory system, where they infect red blood cells (RBCs) and undergo further multiplication and differentiation, a phenomenon referred to as trophic period, producing trophozoites. The enlargement of these trophozoites results into the production of schizonts (Tuteja, 2007). The erythrocytic stage of the *Plasmodium* life cycle is responsible for most malaria associated pathologies including fever and anaemia. CHQ, the drug of choice in the current study exerts anti-malarial activity by targeting this erythrocytic stage of the life cycle. The destruction of RBCs by the parasite is responsible for the reported malaria-associated anaemia. However, it is unclear whether this anaemia is solely due to the parasite or is exacerbated by CHQ**.**

The shizonts that are produced from the erythrocytic stage of the life cycle either further infect more red blood cells or differentiate into sexual forms, macro- or microgametocyte. Ingestion of these microgametoctes by the next feeding *Anophele* mosquito triggers gametogenesis (Tuteja, 2007, Wells *et al.*, 2009). The sexual cycle in the vector is completed by differentiation of some merozoites into male and female gametocytes which subsequently develop into sporozoites (Clark & Schofield, 2000, Mackintosh *et al.*, 2004). The cycle is initiated again when these sporozoites are introduced into the next vertebrate host. Figure 1 illustrates the basic life cycle of all the *Plasmodium* species.

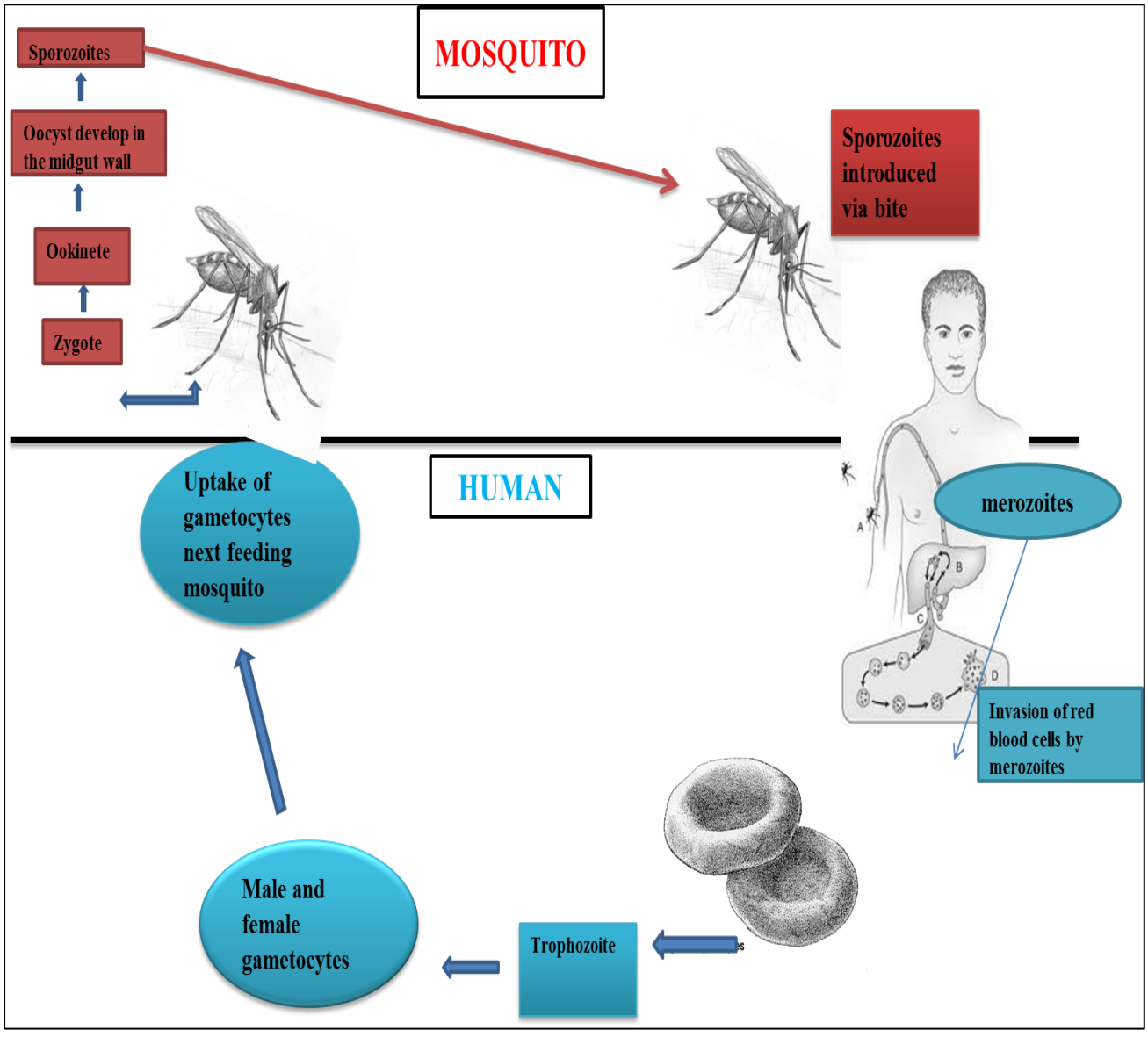

Figure 1: Illustration of the life cycle of malaria parasites of *Plasmodium* spp

The increase RBC destruction by the *Plasmodium* parasite is thought to play a major role in the development of anaemia. However, the destruction of RBCs alone is not adequate to explain the severe anaemia that is reported in malaria patients receiving oral CHQ treatment. Hence, the current study will investigate the possible mechanism for the reported anaemia.

Anaemia is one of the most severe malaria complications and accounts for a significant number of malaria related mortality. The pathophysiology that is associated with this complication is not clearly understood. However, some studies have reported the involvement of both intravascular and extravascular destruction of parasitized and unparasitized RBCs as well as a reduced erythropoiesis (Bjo¨rkman, 2002). In chronic anaemia hypersplenism and dyserthropoiesis are believed to be the main factors causing anaemia, whereas in acute anaemia intravascular is the focal factor (Abdalla *et al.*, 1980). In malaria infection, there is a high rate of RBC destruction by the parasites without any parallel replacement by erythropoiesis. This destruction is mediate through multiple mechanisms including the rupture of RBC, phagocytosis of parasitized and unparasitized RBCs and hypersplenism. During its life cycle, the *Plasmodium* parasite invades RBCs where replication occurs, resulting into the rupturing of RBCs to release schizonts, resulting to the reduction of RBCs (Phillips *et al.*, 1986, Phillips & Pasvol, 1992). The proliferation and hyperactivity of macrophages within the reticuloendothelial system in response to the destruction of RBCs is responsible for the phagocytosis of both parasitized and unparasitized RBCs. This removal of unparasitized RBCs is believed to be one of the mechanisms resulting into persistent anaemia (Phillips & Pasvol, 1992 , Slutsker *et al.*, 1994). Splenic hypertrophy and hypersplenism also contribute to the destruction of RBCs during malaria infection. The spleen plays a pivotal role in the clearance of the *Plasmodium* parasites by filtering parasitized RBCs from the circulation, hence contributing to the early anaemia associated with acute malaria (Angus *et al.*, 1997).

1.2.1. Role of hepcidin in anaemia

Recent evidence suggests that the above mentioned mechanisms alone or in combination do not adequately explain the anaemia of malaria infection (McDevitt *et al.*, 2004). Hepcidin, a recently discovered peptide hormone, is a major regulator of iron metabolism and is thought to play a central role in the anaemia of chronic inflammation (Drakesmith & Prentice, 2012)

The presence of the malaria parasite triggers the host's innate immune system. Also, the release of toxins during the *Plasmodium* parasite's life cycle triggers the release of the pro-inflammatory cytokines, TNF-α, IL-6 and IL-22. Together with type I interferon, these cytokines stimulate hepcidin transcription through STAT3 signalling (Howard *et al.*, 2007). This hepcidin then blocks the release of iron from enterocytes, hepatocytes, and macrophages, leading to hypoferremia and limited iron availability for erythropoiesis (McDevitt *et al.*, 2004). Some studies have suggested that the quinolone ring of CHQ and other quinine related drugs may act as a hapten, and binds with specific RBC proteins to elicit an immune response leading to the destruction of red blood cells, thus aggravating anaemia (Clark & Cowden, 2003). In summary, the exact cause of anaemia in CHQ treated malaria patients still remains unclear. Therefore, there is need to investigate and distinguish between the pathophysiological effects of malaria alone and those of CHQ treatment on parameters such as haematocrit. Haematocrit is the volume percentage of red blood cells in the blood and gives an integral part of the complete blood count. The current study will, therefore, investigate and compare the effects of malaria infection and that of treatment on haematocrit levels in an effort to establish the possible mechanism for the reported malaria associated anaemia.

1.3. Management of malaria

1.3.1. Control of malaria transmission

Vector control is one of the most effective methods used to reduce the transmission of malaria at the community level (WHO, 2013). Two forms of vector control, indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLINs) have been shown to reduce malaria transmission when used against insecticide susceptible mosquito populations. IRS and LLINs reduce the *Anopheles* mosquito's daily survival rate and human biting frequency. However, mosquito resistance to pyrethroids, has been reported in some malaria endemic areas, including Sub-Saharan Africa. This increasing resistance of *Anopheles* mosquitos to pyrethroids has resulted in an increase in malaria related morbidity and mortality rates (WHO, 2013). The increasing number of pyrethroids resistant *Anopheles* mosquitos has led to the increased use of self-administration antimalarial drugs for the treatment and prophylaxis of malaria. The use of these antimalarial drug has resulted in a significant reduction in malaria related morbidity and mortality rates in many tropics including India and Sub-Saharan Africa (WHO, 2013). The next section will discuss some of the anti-malarial drugs

that are used for the management of malaria including CHQ which will be used in the current study for transdermal application.

1.3.2 Drug intervention for the management of malaria

Chemotherapy remains the most effective intervention for the reduction of malaria associated mortality and morbidity rates. The increasing resistance of *P.falciparum* to a number of anti-malarial drugs poses a great challenge in the management of malaria. There are many standard anti-malaria drugs which have been put in place for the management of malaria; including artermisinin derivatives, antifolates and quinine related drugs which can be used as monotherapy or in combination for the treatment of malaria. The following sections discuss the various anti- malarial drugs as well as their mechanisms of action. More often than not, these drugs are associated with inconvenient dosing schedules which lead to patient non-compliance. This patient non-compliance is believed to be one of the factors contributing to the increased number of resistant *Plasmodium* strains. The current study will investigate transdermal drug delivery as an alternative CHQ administration to try and eliminate the inconvenient dosing schedule that is associated with oral CHQ administration.

1.3.2.1 Artemisinin derivatives

Artemisinin also known as qinghaosu; is a natural component isolated from *Artemisia annua*. The plant *Artemisia annua* has been used since ancient times by the Chinese community as a traditional remedy for the treatment of fever. It was not until the early 1970s that the active ingredient, artemisinin, was isolated and the anti-malaria activity of this plant extract against *P. falciparum* demonstrated. A number of artemisinin derivatives have been synthesized and shown to be potent against *P. falciparum* (Meshnick *et al.*, 1996, Meshnick, 2002). Some of these derivatives include artemether, arteether, artesunate and artenimol. The success of artemisinin derived anti-malarial drugs is ascribed to the reactivity of the endoperoxide bridge; a structural feature that is common in all artemisinin derived anti-malarial drugs. The novel structure and mode of action of these artemisinin derivatives has resulted to a reduction in malaria related mortality and morbidity rates (Meshnick *et al.*, 1996). One of the problems with the use of artemisinin drugs is that when used as monotherapies over short periods , the clearance of the malaria parasites from the blood is only temporary in up to 50% of patients (Meshnick *et al.*, 1996). This recurrence is believed to be ascribed to the rapid elimination of

these artemisinin drugs from the body. Hence artemisinin drugs are now used in combination with other antimalarial drugs which have slow elimination rates, such as mefloquine. The combination of a high dose of mefloquine at the end of a full course of artemisinin drug treatment has been shown to produce higher cure rates (Looareesuwan *et al.*, 1994). In addition, due to the increasing reports of artemisinin resistance strains of *P. falciparum*; the WHO has recommended the use of artemisinin derivatives as combination therapies (ACTs) (WHO, 2003). Some of the commonly used ACTs include artesunate-amodiaquine, artemether-lumifantrine and artesunate-mefloquine. Even though these ACTs have shown potency against resistant *P. falciparum*, they are however inaccessible to developing countries due to high cost. An estimate of 48.4 million dollars has been spent for the implementation of ACT worldwide. As a result, quinine drugs such as CHQ remain the mainstay therapy in many African countries.

1.3.2.2 Quinine related drugs

Quinine, extracted from Cinchona bark, has provided the basis for the development of synthetic quinoline-containing antimalarial drugs such as chloroquine (CHQ), primaquine and mefloquine. Quinine is a basic, cinchona alkaloid that belonging to the arylamino alcohol group of drugs (Achan *et al.*, 2011). The exact mechanism by which this anti-malarial drug exerts therapeutic effects remains unclear. Quinine has rapid schizonticidal properties against intra-erythrocytic malaria parasites. This antimalarial drug also possesses gametocytocidal properties against *P. vivax and P. malariae* (Achan *et al.*, 2011) but has no effects against *P. falciparum*. According to the WHO, quinine has a low therapeutic index and is associated with multiple adverse effects (WHO, 2000), including abdominal pain, diarrhoea, and also loss of vision (Achan *et al.*, 2011) which has resulted to the limited use of this anti-malarial drug. Primaquine is an 8-aminoquinoline with schizonticidal activity against pre-erythrocytic liver latent tissue forms of *P. vivax* and *P. ovale* (Fernando *et al.*, 2011). Primaquine is rapidly absorbed in the gastrointestinal tract and peaks in plasma within 1-3 hours following administration. This drug has a short plasma half-life of 4-9 hours and is rapidly excreted in urine (Fernando *et al.*, 2011). Previous studies have shown that primaquine elicits adverse haemolytic effects in glucose-6-phosphate dehydrogenase (G6PD) deficient patients including pregnant women (Amos *et al.*, 2010, Fernando *et al.*, 2011). In addition, the inability of this drug to confer protection against asexual blood stages of *P. falciparum* at it therapeutic doses has resulted in decreased utilization of this drug (Baird &

Hoffman, 2004). As a result CHQ has remained a mainstay therapy in some areas. However the traditional mode of administration, oral, is associated with transiently high plasma CHQ concentrations which in turn elicit adverse effects on blood glucose homeostasis and renal functions. Transdermal drug delivery of CHQ will be investigated as an alternative CHQ administration method. The next sections briefly discuss CHQ, looking at its mechanism of action as well as its effects on blood glucose and renal electrolytes handling.

1.4 Chloroquine (CHQ)

CHQ phosphate is a 4-aminoquinoline antimalarial drug that has shizonticidal properties. This antimalarial drug was ignored for a decade and was considered to be too toxic for human use. However, during the early 90s clinical trials for anti-malarial drugs development were carried out and showed unequivocally that CHQ has a significant therapeutic value as an anti-malarial drug. In 1947, CHQ was introduced into clinical practice for the prophylactic treatment of malaria. The great success of this drug is believed to be attributed to its mechanism of action.

1.4.2 Mechanism of action of chloroquine

CHQ's efficacy is speculated to lie in this drug's ability to interrupt the detoxification of haematin in malaria parasites as they grow within their host's red blood cells. Haematin is released in large amounts as the parasite consumes and digests haemoglobin within the digestive food vacuoles (Zhang *et al.*, 1999). Haematin is highly toxic to the malaria parasite and is normally detoxified by polymerization into innocuous crystals of haemozoin pigment and perhaps also by a glutathione-mediated process (Zhang *et al.*, 1999, Wellems & Plowe, 2001). CHQ exerts antimalarial activity by interfering with the process of haematin detoxification. This 4-aminoquinoline binds with haematin in its μ- oxodimer form and adsorbs to the growing faces of the hemozoin crystals disrupting the detoxification process, thus poisoning the parasite (Zhang *et al.*, 1999). The result is the generation of toxic CHQ-haeme complexes as well as accumulation of free haem moieties within the parasite's food vacuole. The accumulation of these moieties results in RBC lysis, eventually killing the malaria parasite (Zhang *et al.*, 1999)

The reasonable cost and rapid onset of action of CHQ has resulted to the continuous use of this drug despite of the reported resistance. However, over the years CHQ use has decline due to a growing number of CHQ-resistant strains of *P. falciparum.* The decrease in the CHQ efficacy is thought to be attributed to a number of factors which are associated with the conventional route of administration (Musabayane *et al*., 2003). The conventional route of CHQ administration involves oral administration of tablets. This oral dosing schedule involves the administration of 4 tablets (with 250mg of chloroquine diphosphate in each tablet) upon presentation of the disease, 2 tablets taken 6 – 8 hours later, and then 2 tablets per day for the succeeding 2 days, adding up to a total of 2500mg of chloroquine diphosphate. Patient noncompliance during CHQ treatment is one of the factors that is thought to cause therapeutic failure of this drug (Musabayane *et al.*, 2003). This patient non-compliance often results in incomplete treatments, which is believed to have led to the growing number of CHQ-resistant strains of *P. falciparum* in Sub-Saharan Africa (White, 2004). In addition, when administered orally, CHQ is susceptible to gastrointestinal degradation as well as first-pass metabolism by the liver; as a result high initial CHQ concentrations are required to bring therapeutic effects. The use of high CHQ concentrations leads to accumulation of CHQ in various tissues (Gustafsson *et al.*, 1983), which subsequently interferes with a number of physiological functions including glucose homeostasis and renal functions. However, the exploitation of alternative novel CHQ delivery routes could eliminate the inconvenient dosing schedule associated with the oral route by providing sustained controlled CHQ release over a long period of time and therefore avoiding high repetitive doses. Later sections will discuss the transdermal drug delivery as an alternative CHQ route of administration to oral delivery.

1.4.3 Chloroquine absorption, metabolism and excretion

CHQ is slowly eliminated from the body and hence accumulates in certain organs and tissues of the body. Following oral administration, maximum plasma concentrations of CHQ are attained within1-4 hours, indicating the drug's rapid absorption (Gustafsson *et al.*, 1983). About 70% of the administered dose of CHQ is excreted unchanged in the urine and the parent drug can still be detected in the urine for up to 120 days following a single 300 mg dose in humans (Pussard & Verdier, 1994). The high tissue affinity of CHQ is supported by the detection of high concentrations of this drug in organs following oral and intravenous

infusion of the drug (Gustafsson *et al.*, 1983). After administration CHQ is swiftly dealkylated through cytochrome P450 enzymes (CYP) into the pharmacologically active mono-desethylchloroquine and bisdesethylchloroquine (Pussard & Verdier, 1994, Durcharme & Farinotti, 1996). Mono-desethylCHQ is the main metabolite of CHQ and has been shown to possess anti-malarial activity against CHQ-susceptible *P. falciparum* as the parent compound (Aderounmu, 1984). The ability of CHQ to bind and accumulate in various tissues following oral administration is thought to be one of the main factors responsible for the reported CHQ-induced disturbances in blood glucose homeostasis and impaired renal electrolytes handling. Therefore, there is a need of novel CHQ delivery routes, such as transdermal drug delivery systems, which will avoid the transiently high plasma CHQ concentrations observed following oral CHQ administration. In addition, the pathological effects of malaria on these parameters are unclear. Currently, there is no literature evidence that distinguishes the pathophysiological effects of malaria from that of CHQ in malaria patients. The next section discusses the some of the speculated pathophysiological effects of malaria and oral CHQ on glucose homeostasis, renal function.

1.5 Adverse effects of CHQ or malaria parasites

In the unprotonated form, CHQ is relatively lipophilic and is therefore absorbed and accumulated in cell plasma membranes prior to cell entry (Mellman, 1986). Chloroquine accumulates in vacuoles, causing the neutralization of protons. The neutralization of these protons by CHQ results to in an increase in vacuolar pH (Poole & Ohkuma, 1981). Increase in intra-vacuolar pH is associated with the disruption of vacuole associated physiological processes, including the deactivation enzymes such as nucleases, proteases, glycosidases, lipases and phosphatases (Lenz & Holzer, 1984). The transiently high plasma CHQ concentrations following oral administration are associated with the accumulation of this drug in various organs including the kidneys, liver and heart where the drug elicits adverse effects such as impaired kidney function and impaired glucose homeostasis. However, it is unclear whether these adverse effects are solely due to CHQ or whether the malaria parasite also exerts pathophysiological effects on these systems. At present, no studies have been done to distinguish between the pathophysiological effects of CHQ and malaria on blood glucose and renal function. The next sections will discuss some of the possible effects of CHQ and malaria on blood glucose, renal function and blood pressure.

1.5.1 Effects of malaria on glucose homeostasis

Hypoglycaemia has been reported in tropical malaria patients (Jarzyna *et al.*, 2001). Multiple mechanisms through which the malaria parasites may precipitate hypoglycaemia in malaria patients have been proposed; including the inhibition of gluconeogenesis and anaerobic glycolysis (White *et al.*, 1987, Taylor *et al.*, 1988). The utilization of the host's glucose stores by the malaria parasites is another factor that is believed to contribute to hypoglycaemia (White *et al.*, 1983). Due to the high demand of glucose by the parasites, there is an increase exhaustion of the host's hepatic glycogen stores to meet these demands, resulting reduced blood glucose levels (White *et al.*, 1983). However, there are no studies which have been conducted to support these proposed theories. Hence the current study investigated the effect of the *Plasmodium* parasite on blood glucose homeostasis to try and establish the mechanism for the reported hypoglycaemia in malaria patients.

1.5.2 Effects of CHQ on glucose homeostasis

Chloroquine has been reported to cause hypoglycaemia following oral administration (Jarzyna *et al.*, 2001). Numerous studies suggest that CHQ may induce hypoglycaemia through multiple unclear mechanisms. Some literature evidence suggests that the CHQ-induced hypoglycaemia is mediated through increased insulin secretion (Davis, 1997) or decreased insulin degradation (Knutson *et al.*, 1985). Chloroquine is a diprotic base and is rapidly accumulated in acidic lysosomes and endosomes thereby elevating lysosomal pH. This increase in lysosomal pH compromises the optimal activity of lysosomal hydrolases that are responsible for the degradation of insulin, causing an increase in plasma insulin concentration (Knutson *et al.*, 1985). Jarzyna and colleague (1997) reported the inhibition of enzymes that are involved in gluconeogenesis following oral CHQ administration. These enzymes include glutamate dehydrogenase, a key enzyme in amino acid metabolism in the liver and kidney (Jarzyna *et al.*, 1997, Jarzyna *et al.*, 2001). The inhibition of this enzyme in turn results in a decrease in hepatic glucose output, which precipitate hypoglycaemia. The accumulation of CHQ in the liver following oral administration has been reported to cause hepatocellular damage. This accumulation is associated with a decreased pyruvate transport into the mitochondria as well as the inhibition of glucose-6-phosphatase, which is required for gluconeogenesis (Jarzyna *et al.*, 2001). In summary, it is unclear whether the reported

changes in blood glucose levels in malaria patients is caused by the parasite or oral administration of CHQ . Accordingly, the present study investigated the pathophysiological effects of malaria and CHQ on blood glucose to try and establish the main cause of the reported changes in blood glucose homeostasis.

1.5.3 Effects of malaria on renal function

Mild hyponatraemia, hyperkalaemia and acidosis have been reported in patients with complicated *P. falciparum* malaria (Etim *et al.*, 2011). The reported hyperkalaemia is speculated to be linked to the increased acidosis that is associated with increased protein catabolism. Increased metabolic acidosis results in increased availability of hydrogen ions (H^+), these H^+ are involved in renal tubule sodium exchange at the expense of potassium (K^+) ions, resulting into increase potassium retention (Etim *et al.*, 2011). The increased release of K^+ from RBCs during haemolysis is also thought to increase plasma K^+ concentrations, resulting into hyperkalaemia. Constriction of renal vascular bed as well as splachnic blood vessels has been reported in malaria associated renal failure (Das, 2008.). This constriction of renal vascular bed more often than not leads to renal ischaenia. This ischaemia causes a reduction in urine volume, GFR and urinary sodium excretion (Elsheikha & Sheashaa, 2007, Das, 2008.). Parasitized red cells are sticky and tend to adhere to adjacent unparasitized erythrocytes and the capillary endothelium. This in turn, results in the formation of intravascular clumps that impede the renal microcirculation of internal organs (Elsheikha & Sheashaa, 2007, Das, 2008.).

1.5.4 Effects of CHQ on renal function

Current evidence suggests that CHQ may influence kidney function when administered acutely or chronically in rats (Musabayane *et al.*, 1993). High plasma CHQ concentrations have been observed 1-4 hour following administration of the drug (Gustafsson *et al.*, 1983). About 51% of this CHQ is cleared by the kidney unchanged (McChesney *et al*., 1966). However, a small fraction of it accumulates in epithelia cells of the kidneys (Gustafsson *et al.*, 1983). This deposition of CHQ in the kidneys is reported to alter kidney function. In addition, some of the administered CHQ is deposited in in the heart (Baguet & Fabre, 1999) where CHQ exerts antiarrythmic actions to increase heart rate (Dondo & Mubagwa, 1990). The increase in heart rate is speculated to alter the perfusion pressure of the kidney, and amend renal haemodynamics as well as electrolyte handling. We have reported an increase in

renal sodium (Na^+) excretion without any changes in urine flow rate following acute intravenous CHQ administration (Musabayane *et al.*, 1993). This increase in Na^+ cannot be explained by changes in aldosterone, as no changes in this hormone were recorded. The mechanism for this natriuresis is thought to be attributed to CHQ induced increase in plasma AVP (Musabayane *et al.*, 1993). Chronic administration of CHQ has been reported to cause renal Na^+ retention possibly via increased plasma aldosterone concentration and reduced glomerular filtration rate (GFR) (Musabayane *et al.*, 1994 , Musabayane *et al.*, 2000b). The increased Na^+ retention following chronic CHQ administration may also be accredited to the CHQ-induced changes in the kidney (Musabayane *et al.*, 2000b)

Chloroquine has been previously reported to increase plasma the hormone, arginine vasopressin (AVP) following intravenous infusion. This hormone plays an imperative role in water homeostasis within the body, and is thus vital for the regulation of blood pressure. The CHQ induced increase in plasma AVP is thought to be linked to the action of CHQ on the pathway through which AVP acts to increase membrane permeability of the renal tubules(Musabayane *et al.*, 2000). We have shown that orally administered CHQ inhibits the activation of adenylate cyclase (Musabayane *et al.*, 2000). Adenylate cyclase is responsible for the activation of adenosine 3', 5' – cyclic monophosphate (cAMP). This monophosphate consequently phosphorylates aquaporin-2, resulting to the integration of aquaporins into the medullary collecting ducts and collecting tubules, ultimately causing an increase in water permeability (Boone & Deen, 2008). The inhibition of adenylate cyclase by oral CHQ results in reduced incorporation of aquaporins with no change in urine flow rate even though plasma AVP concentrations has been increased (Musabayane *et al.*, 2000). The CHQ induced increase in AVP is believed to be mediated through pathways which involve nitric oxide. It is thought that oral administration of CHQ results in the activation of the enzyme, nitric oxide synthase within the kidney. In turn, NOS stimulates the secretion of AVP (Ahmed *et al.*, 2003). This AVP consequently causes an increase in Na^+ excretion.

Both oral and intravenous administrations of CHQ were associated with compromised renal function attributed to changes in renal hormones as well as kidney morphology. The current study will investigate the suitability of transdermal drug delivery route for the administration of CHQ. In addition it is unclear whether CHQ is the main cause of these changes in renal functions as the effects of the malaria parasites on renal function are unclear. Accordingly,

the study will investigate the effects of malaria alone on renal function and that of CHQ on renal function.

1.6 Methods of drug delivery

The conventional route for the administration of CHQ involves oral administration of tablets. In the past years this method of CHQ delivery has been successful in eradicating the malaria parasite during infection. However, oral administration of CHQ is associated with a number of disadvantages that have been previously described. When administered orally, CHQ has been shown to accumulate in epithelial cells of various tissues, such as the heart, kidney, spleen, adrenal glands and liver, disrupting their functioning. Due to the accumulation of CHQ in the circulation when administered orally, a number of vital organs such as the heart, kidney and liver, are negatively affected. This delivery system is also associated with an inconvenient dosing schedule. Hence the current study will investigate a novel CHQ delivery system, which will eliminate the inconvenient dosing schedule that is associated with oral administration and mask bitterness of CHQ. This new delivery method is also envisaged to minimize the toxicity of the drug observed in oral administration, by reducing the amount of deposition of the drug in various tissues. In our laboratory we are focusing on transdermal drug delivery system. We have previously shown that oral administration of amidated CHQ beads formulation is able to sustain control CHQ release into the blood stream (Munjeri *et al.*, 1998). In addition, we have successfully delivered CHQ topically into the rat via the use of an amidated pectin hydrogel matrix patch as assessed by changes in renal Na^+ changes (Musabayane *et al.*, 2003). However, plasma CHQ concentrations following transdermal CHQ administration has never been measured. Also, the effect of this pectin CHQ matrix patch on parasitaemia has not yet been demonstrated. Accordingly, this study will investigate the effects of transdermal delivery of CHQ on *P. berghei* infected rats, and evaluate plasma CHQ concentrations following transdermal application.

1.6.1 Transdermal drug delivery

Transdermal drug delivery (TDD) systems are topically administered drugs in the form of patches. These systems deliver drugs for systemic effects at a predetermined and controlled rate (Chien, 1992, Prausnitz & Langer, 2008). A number of drugs have been successfully delivered using transdermal delivery system, including nicotine. This delivery system has a

number of advantages when compared to oral and intravenous administration. When administered orally drugs are susceptible to first pass degradation by the liver as well as gastrointestinal metabolism (Chien, 1992, Prausnitz & Langer, 2008). As a result of this degradation high drug concentrations are required for therapeutic effects (Prausnitz & Langer, 2008). In transdermal delivery system, there is no first pass metabolism by the liver since the drug is delivered directly into the systemic circulation. Since there is no first pass metabolism by the liver low drug concentrations are required. The great success of this system is lies in its ability to provide a sustained controlled release (Musabayane *et al.*, 2003) and thus presenting drug deposition in tissues. When compared to hypodermic injections, which are painful, generate dangerous waste and pose a risk of disease transmission by needle re-use (Prausnitz & Langer, 2008), transdermal delivery proves to be a better suited method for drug delivery.

TDD proves to be a much more convenient route for the administration of CHQ. However, this system is limited by the skin outer layer, the stratum corneum. The stratum corneum is physiologically responsible for the vital barrier function of the skin and does not allow drugs to spontaneously pass into the systemic circulation. The next section will discuss the composition of the stratum corneum as how it hinders drug transport into the systemic circulation for TDD systems.

1.7 Physiology of the skin

The skin is the largest organ of the human body and performs multiple vital functions which include forming a protective barrier against external chemicals, pathogens as well as preventing excessive body water loss. Skin is an important site of drug application for both local and systemic effects (Pathan & Setty, 2009). The skin is composed primarily of three structures, the epidermis which is the outermost layer, the dermis, and subcutaneous tissue (Kanitakis, 2002) .

The epidermis is made up mainly of keratinocytes and dendritic cells (Kolarsick *et al.*, 2011). However, keratinocytes form a large part of the epidermis. This outermost layer is commonly divided into four layers, the stratum germinativum, the stratum spinosum, the stratum granulosum and the stratum corneum (Murphy, 1997). Of all the various layers of the skin, the stratum corneum is the rate-limiting barrier to topical application of drugs. The stratum

corneum confers mechanical protection to the underlying epidermis and invasion by foreign substances by acting as the main barrier for diffusion of the permeants (Jackson *et al.*, 1993). This outermost layer of the skin is made up of 18-21 hexagonal cells, the corneocytes. The corneocytes are rich in keratin and are scattered within crystalline lamellar lipid matrix to adopt a bricks and mortar arrangement (Elias, 1983). About 10% of this layer is made up of extracellular lipid. Literature evidence suggests that the barrier function of the skin is ascribed to the lamellar lipids that are synthesized in the granular layer. These lipids are successively organized into the lipid bilayer domains of the stratum corneum. The lipid domains of the stratum corneum are the ones that pose a challenge for transdermal drug delivery as they hinder spontaneous penetration of hydrophilic drugs. The transport of hydrophilic and charged molecules into the systemic circulation is hindered by the lipid-rich nature of the stratum corneum as well as the low water content (20%) (Mehta, 2004).

The rate at which drugs are transported across the stratum corneum is not only determined by solubility of the drug, but is also directly proportional to the oil/water partition coefficient, drug concentration in the formulation vehicle as well as the surface area of the skin exposed (Mehta, 2004). Current evidence indicates that the thickness of the stratum corneum varies in different regions of the body and may affect drug transport. The stratum corneum is thickest in the plantar and palmar regions and thinner in the postauricular, axillary, and scalp regions of the body (Mehta, 2004). Thus drug transport may be limited in the regions such as the plantar and palmar regions. In an effort to maximise drug flux for TDD systems, penetration enhancement methods have been investigated. The next section discusses various penetration enhancers including dimethyl sulphoxide (DMSO) which will be used in the present study to enhance the transdermal delivery of CHQ.

1.8.Penetration enhancers

Penetration enhancers refer to substances that aid the absorption of molecules/drugs through the skin. For a penetration enhancer to be considered ideal certain criteria which include pharmacological properties, inertness, non-toxicity, nonirritating, nonallergenic, compatibility with the drug , odourless, and good solvent properties must be met (Finnin & Morgan, 1999, Sinha & Kaur, 2000). The permeation of drugs through the skin can be enhanced through both chemical penetration enhancement and physical methods. However

for the purpose of this study we shall be focusing on some chemical penetration enhancers as well as their plausible mechanisms of action.

1.8.1. Sulphoxides

Dimethyl sulphoxide (DMSO) is the earliest and most potent penetration enhancer belonging to the category of sulphoxides. This permeation enhancer is a very strong aprotic solvent capable of hydrogen bonding with itself rather than water (Williams & Barry, 2012). Although DMSO is a good enhancer, this sulphoxide has concentration-dependant effects. At high concentrations, DMSO can cause erythema and wheals of the stratum corneum and may denature proteins (Williams & Barry, 2012). This enhancer uses multiple, complex mechanisms to aid the permeation of drugs. DMSO alters the stratum corneum's keratin from alpha-helical to β-sheet conformation (Barry, 2001, Williams & Barry, 2012). The physiochemical structure of DMSO allows for the interaction of this molecule with the head groups of the lipid bilayer. This DMSO-lipid bilayer interaction alters packing of the lipid bilayer. In addition, DMSO penetrates and translocates forming microcavities with the lipid bilayer which increase volume available for drug diffusion.

Dimethylacetamide (DMAC) and dimethylformamide (DMF) are chemical enhancers which are chemically related to DMSO. Similar to DMSO, both DMAC and DMF have a wide range of penetration enhancing activities (Williams & Barry, 2012). Like DMSO, DMF has been shown to cause irreversible changes to the stratum corneum at high concentrations (Williams & Barry, 2012). Another DMSO structural analogue that has been synthesized is decymethylsulphoxide (DCMS). This analogue has ability to act reversibly on the stratum corneum. However, like DMSO, DCMS has concentration dependent effects. Literature evidence indicates that DCMS is more potent in the permeation of hydrophilic permeant and is less effective at promoting transdermal delivery of lipophilic drugs (Barry, 2001, Williams & Barry, 2012). Hence DMSO remains a potent sulphoxide penetration enhancer. In the present study, DMSO was used in combination with an antioxidant, vitamin E, as a penetration formulation for transdermal delivery of CHQ.

1.8.2. Fatty acids

Numerous fatty acids have been used as permeation enhancers. Scientific evidence has shown that unsaturated fatty acids are much more effective in permeating the skin when compared to saturated fatty acids (Chi *et al.*, 1995). Oleic acid and sodium oleate are amongst many penetration enhancers classified under fatty acids. According to Williams and colleagues, fatty acids with unsaturated alkyl chain lengths of around C_{18} attached to a polar head group yield optimum enhancers (Williams & Barry, 2012). Oleic acid has been shown to enhance penetration of a number of drugs at very low concentrations (Sintov *et al.*, 1999). This permeation enhancer aids skin permeation by interacting with and modifying the lipid domains of the stratum corneum. In addition to this, oleic acid has an ability to form pools and exist as a separate phase. These pools offer permeability defects within the lipid bilayer thus allowing for the permeation of hydrophilic drugs (Tanojo *et al.*, 1997, Williams & Barry, 2012).

1.8.3. Antioxidants

Antioxidants are another class of potent penetration enhancers. One of the commonly used antioxidant is vitamin E. Vitamin E intercalates within the lipid bilayer and distorts the gel phase lipids, decreasing the gel state of phospholipid membrane, causing permeation (Thiele & Ekanayake-Mudiyanselage, 2007). In addition to skin permeation enhancement, Vitamin E confers protection to the skin due to the antioxidative properties of this vitamin, preventing skin damage. Vitamin E has been previously used in our laboratory as an antioxidant for transdermal drug delivery to thwart physiological damage to the skin. This antioxidant will be used in the current study to enhance CHQ permeation and prevent skin damage.

Transdermal drug delivery has many attractions, including increased patient compliance and avoidance of gastrointestinal disturbances and first-pass metabolism of the drug. The stratum corneum is one of the limiting factors for the use of transdermal delivery as an alternative to other conventional drug delivery routes. However, the development penetration enhancers have played a substantial role in improving transdermal drug delivery systems and their pharmaceutical use. Both penetration enhancers and transdermal delivery polymers form a vast part of transdermal drug delivery systems. The next section will discuss transdermal polymers, focusing solely on pectin which will be used in the present study. Oral administration of pectin has been reported sustain control CHQ release (Munjeri *et al.*, 1998).

We, therefore, speculate that transdermal application of CHQ via the pectin CHQ patch will sustain controlled release of CHQ into the blood stream.

1.9.Transdermal delivery polymers

Polymers are the backbone of a transdermal drug delivery system and form a big part of these systems. The following section will focus on the chemistry of pectin as a transdermal drug delivery polymer. Pectin is a non-toxic, water soluble gel-forming polysaccharide that has been widely used in many transdermal delivery systems due to its virtuous gelling properties which will be discussed in details in the next section.

1.9.1. Chemistry of pectin

Pectin is a naturally occurring polysaccharide that has gained increasing application in pharmaceutical and biotechnology industries. This biopolymer has been used successfully for many years in the food and beverage industry as a gelling agent and a colloidal stabilizer (Sriamornsak, 2003). Pectin is a methylated ester of polygalacturonic acid. This polymer is commercially extracted from citrus peels and apple pomace under mildly acidic conditions (Sriamornsak, 2006). The composition and structure of pectin are poorly understood. Pectin consists mainly of D-galacturonic acid (GalA) units which are joined in chains by means of (1-4) glycosidic linkage. These uronic acids have carboxyl groups, some of which are naturally present as methyl esters and others which are commercially treated with ammonia to produce carboxamide groups. In addition to the galacturonan segments, neutral sugars are also present. Rhamnose (Rha) is a minor component of the pectin backbone and introduces a kink into the straight chain and other neutral sugars such as arabinose, galactose and xylose occur as side chains (Oakenfull, 1991).

The ability of pectins to form gels depends on the molecular size and degree of esterification (DE). The ratio of esterified GalA groups to total GalA groups is referred to as the DE. This DE ratio plays a major role in the classification of pectins (Sriamornsak, 2006). The polygalacturonic acid chain is partly esterified with methyl groups and the free acid groups may be partly or fully neutralised with sodium, potassium or ammonium (Sriamornsak, 2003). The degree of esterification of pectin is dependent upon the species, tissue and the maturity of the source of pectin (Sriamornsak, 2003). Generally, tissue pectins range between

60-90% DE. Two classes of pectin have been identified, the high methoxyl pectins and the low methoxyl pectins (LM). The low methoxy pectins can either be the conventionally demethylated or amidated molecules (Sriamornsak, 2003). The DE values for high methoxyl generally range between 60-70%, and those of low methoxyl range between 20-40%. Both low methoxyl and high have different gelling mechanisms. High methoxylated pectin requires a pH within narrow range, that is, around 3.0 in order to form gels. In addition, high methoxylated pectin gels are thermally reversible and frequently contain a dispersion agent such as dextrose to prevent lumping (Sriamornsak, 2003). Low methoxylated pectin produce gels independent of sugar content, and are less sensitive to pH compared to high methoxyl pectin.

The rate at which gelation of pectin takes place is affected by the degree of esterification, a higher DE causes more rapid setting. LM-pectins require the presence of divalent cations such as calcium for proper gel formation. The mechanism of LM-pectin gelation relies mainly on what is referred to as the 'egg-box' model (Grant, 1973) . This mechanism involves the formation of junction zones created by the ordered, side-by-side associations of galacturonans, whereby specific sequences of GalA monomer in parallel or adjacent chains are linked intermolecularly through electrostatic and ionic bonding of carboxyl groups. In addition, amidation increases or improves the gelling ability of low methoxy pectin. (May, 1990), reported that amidated pectins require less calcium to gel and that this pectin is less susceptible to precipitation at high calcium concentrations. During gelation of amidated low methoxyl pectins, ionic interaction between galacturonic acid residues and hydrogen bonding between amidated galacturonic acid residues aid the gelation of the pectin (Sriamornsak, 2002). These interactions result in the formation of a net-like cross-linked pectin molecule (Sriamornsak, 2002). The cross-linkages formed by ionic bonds between the carboxyls are also involved in the gelling of amidated pectins and form stronger bonds, producing a brittle, less elastic. This cross linking is of vital importance in the entrapping of drugs for TDD.

The natural occurring polysaccharide, pectin, has a wide spectrum of pharmaceutical uses. These include cations poisoning prophylaxis, reduction of blood cholesterol levels and in diet(Wilson & Dietschy, 1974). In the current study, amidated pectin of lower DE will be used to provide a sustained controlled release of CHQ delivery into the systemic circulation and avoid gastrointestinal and hepatic degradation of CHQ. Both the ionic and hydrogen interaction ensure the cross linking and therefore gelling of pectins. In pectins of lower DE,

the addition of the divalent cation, calcium, increases the probability of the formation of cross-links with a given amount of calcium.

1.10 Basis of the present study

We speculated that use of pectin-CHQ matrix patch formulation may provide slow sustained release of therapeutic CHQ doses which clear the malaria parasites in the systemic circulation. We also envisaged that the sustained, slowly released CHQ concentration from the pectin-CHQ matrix patch will avert the avert the adverse effects oral CHQ on blood glucose homeostasis and renal fluid and electrolyte handling of *P. berghei*-infected rats

1.11.Aims/ Objectives of the study

1.11.1. Primary aim of the study

The present study was designed to develop a novel CHQ-formulation that delivers sustained slow CHQ release into the systemic circulation. We also investigated the ability of this CHQ formulation to clear the malaria parasites in *Plasmodium berghei*-infected male Sprague-Dawley rats.

1.11.2. Secondary aims

The secondary aims of the study were to:

- Investigate the ability of the pectin CHQ matrix patch to sustain plasma CHQ concentrations
- The other objective of the study was to investigate and distinguish between the patho-physiological effects of malaria parasites and CHQ treatment on blood glucose homeostasis and renal fluid and electrolyte handling in rats.
- Evaluate the effects of the transdermal delivery of CHQ on selected renal functions and on blood glucose concentration in malaria infected rats.

CHAPTER 2

MATERIALS AND METHODS

2.1. Drugs and chemicals

Drugs and chemicals were sourced as indicated:

Amidated pectin **(**Herbstreith and Fox KG, Neuenburg, Germany**);** chloroquine diphosphate ($C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$), sigmacote, dimethyl sulphoxide (DMSO), Giemsa stain and May-Grunwald solution (Sigma-Aldrich Chemical Company, St Louis, Missouri, USA); calcium chloride ($CaCl_2$), potassium hydroxide (KOH), sodium sulphate (Na_2SO_4), sodium hydroxide (NaOH), 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, Dorset, England), halothane (Fluorothane®, AstraZeneca Pharmaceuticals (PTY) LTD, Johannesburg, South Africa) and Arg^8-Vasopressin ELISA Kit® (Abcam, Cambridge, Massachusetts, USA) . All chemical reagents were of analytical grade.

2.2. Animals

Male Sprague-Dawley rats (90-120 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.

2.3. Ethical consideration

Ethical clearance was obtained from the University of KwaZulu-Natal's Ethics committee (References 088/12 Animal and 057/13 Animal, see Appendices I and II).

2.4. Experimental design

Short-term effects of transdermally applied pectin-formulations and oral CHQ treatment, on parasitaemia, blood glucose concentration and renal function were monitored in separate groups of non-infected and *P. berghei* infected male Sprague-Dawley rats.

2.5. Methods

2.5.1. Preparation of pectin-CHQ matrix patches

Low methoxyl amidated pectin with a degree of esterification (DE) of 23% and an amidation of 24% was used for the preparation of the CHQ patches. This type of pectin has an ability to form gels independent of sugar content, and is less sensitive to pH compared to high methoxyl pectins. Pectin-CHQ hydrogel matrix patches were prepared using a protocol well-established in our laboratory (Musabayane *et al.*, 2003) with slight modifications. Patches were prepared by dissolving various amounts of CHQ (5g and 10g) and pectin (4.4g) in separate beakers containing 110 mL de-ionised water. The beakers were then placed in a water bath at 37°C and agitated at 38 x G using a mixer (Heidolph instruments GmbH & Co. KG, Schwabach, Germany) for 15 minutes**.** Following agitation, DMSO (3.3 mL) was added and mixed for 5 minutes. Subsequently, vitamin E (1.65 mL) and eucalyptus oil (1.65 mL) were added to the mixture. Following 1½ h of further mixing and agitation an aliquot, of the mixture (11 mL) was transferred to a petri dish and frozen at -5^{o}C for 18 hours. After freezing, a 2% $CaCl_2$ solution (1.5mL) was added on top of the frozen pectin and then left to stand at room temperature (±25^{o} C) for 10 min to allow for cross linking. The patches were stored at 4^{o}C until use.

2.5.2. Determination of percentage CHQ incorporation

Pectin CHQ matrix patches with known mean surface area of 4.2 ± 1.5 mm^2, (n=6) were dissolved in 100 mL of Sorensen buffer of pH 7.4 overnight. A volume of 2 mL of the final mixture was drawn for spectrophotometric analysis of CHQ (Ultraviolet Novaspec II spectrophotometer (Biochrom LTD, Cambridge, England). The blank contained CHQ-free pectin hydrogel patches dissolved in Sorensen buffer.

2.6. Induction of malaria

A CHQ susceptible strain of *P. berghei* was used for the induction of malaria. The *P. berghei* parasite was supplied by Professor Peter Smith (University of Cape Town, Division of Clinical Pharmacology, South Africa).This *Plasmodium* parasite is one of the four species of malaria parasites that infects murine rodents of West Africa (Cohuet et al., 2006). *P. berghei* has been used in many studies as an experimental model for malaria (Bagot *et al.*, 2002, Goodman *et al.*, 2013). The *P .berghei's* life cycle is similar to that of human infecting *Plasmodium* parasite, making *P. berghei* a good experimental model to study human malaria. Malaria was induced in male Sprague-Dawley rats via a single intra-peritoneal injection of *P. berghei* (10^5 parasitized red blood cells). (Gumede *et al.*, 2003). Successful malaria induction was confirmed by microscopic examination of Giemsa stained thin smears of the tail blood. Percentage parasitaemia ranging from 15 - 20% was considered as a stable malaria state before commencing any experimental procedures.

2.7. Effects of oral and transdermal CHQ treatments

To evaluate the short-term effects of transdermally applied pectin-CHQ patch, a CHQ patch containing 56 mg was used. This dose was selected based on our preliminary studies.

The studies carried out over a period of 3 weeks divided into pre-treatment (days 0-7), treatment (days 8-12) and post treatment (days 13-21) periods. To distinguish the effects of CHQ treatment and malaria infection on blood glucose concentration and renal function, separate groups of control and CHQ treated non-malaria and malaria infected rats were used. The animals were housed individually in Makrolon polycarbonate metabolic cages (Techniplasts, Labotec, South Africa) at the Biomedical Resource Unit, University of KwaZulu- Natal.

The effects of oral CHQ treatment (30mg), twice daily, 8 hours apart and a once off topical application of the pectin CHQ matrix patch (28mg) were compared in non-infected and *P. berghei*-infected rats. These doses were calculated based on current clinical doses for the treatment of malaria. The orally treated group was given CHQ diphosphate solution (30mg) twice daily at 09h00 and 17h00 by means of a ball-tipped, 18-gauge gavage needle (Kyron Laboratories (Pty) LTD, Benrose, South Africa) attached to a 1 ml syringe, for 5 consecutive days. For transdermal application of CHQ, the animals were divided into separate groups

treated with pectin CHQ matrix patches containing various amounts of CHQ (28, 56 and112 mg) respectively. On the first day of the treatment period, pectin CHQ matrix patches were applied at 9h00. Prior to treatment, the rats were shaved on their dorsal region for the application of the pectin CHQ matrix patch. The patch was secured in place with adhesive hydrofilm (BSN Medical, Pinetown, South Africa) (See Figure 2). Parasite density was monitored daily at 9h00, in all groups of animals by microscopic examination of Giemsa stained thin smears.).

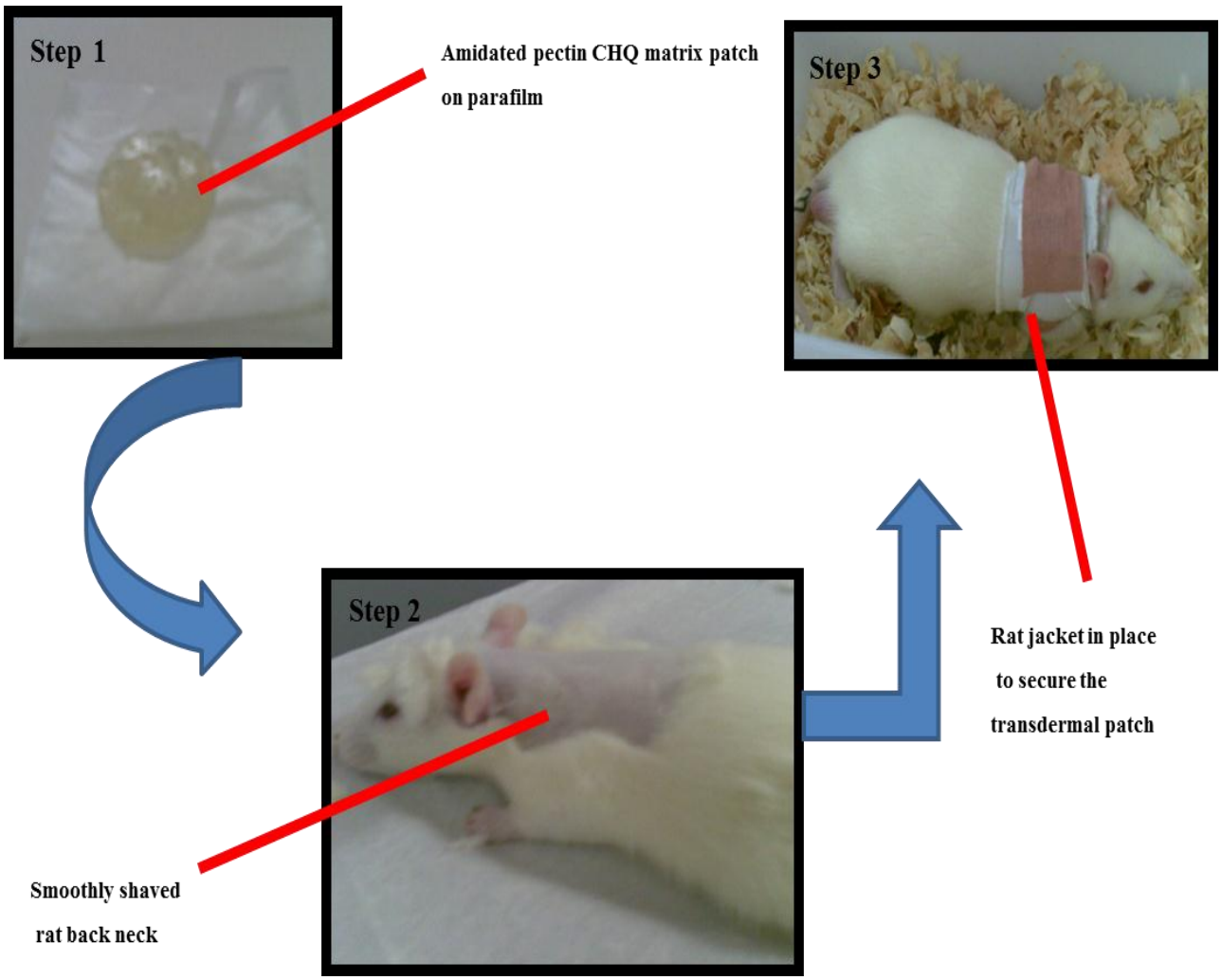

Figure 2: Transdermal application of the pectin-CHQ matrix patch on a shaved rat back neck.

2.7.1. Parasitaemia monitoring

Rats were monitored for % parasitaemia from the day of infection and days of survival relative to the control. Parasitaemia was scored on Giemsa-stained tail-blood films. Briefly, a small incision at the tip of the rat tail was made followed by placing a small drop of blood on a microscope slide. The blood was smoothly and uniformly smeared using a second microscope slide. The blood film on the slide was air-dried, fixed in 90 % methanol for 30 seconds and then stained in Giemsa stain for 20 minutes. Finally, the stained slide was air-dried and viewed under a microscope (Olympus cooperation, Tokyo, Japan) with a x50–x100 oil immersion objective (Olympus cooperation, Tokyo, Japan). Parasite density was calculated as a percentage of infected RBCs. This was achieved by counting the total number of red blood cells and the parasitized red blood cells in 5 microscope fields.

$$\% \text{ parasitaemia} = \frac{\text{Total parasite count}}{\text{Total RBC count}} \times 100$$

2.7.2. Effects of CHQ on blood glucose and physico-metabolic parameters

Body weights, amounts of water, and food consumed were measured in control and treated animals 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, (OneTouch select Test Strips, LifeScan®, Zug, Switzerland). The 24 h urine output, urinary electrolytes (Na^+, K^+), urea and creatinine were also measured (See section 2.9.2).

2.7.3. Effects of CHQ on mean arterial pressure (MAP)

Mean arterial pressure was monitored every third day at 09h00 during the pre-treatment and post-treatment periods. However, during the treatment period MAP was monitored daily in Sprague Dawley rats using the non-invasive tail cuff method (IITC Life Sciences Inc., Woodland Hills, California, USA) as previously described (Musabayane *et al.*, 2007). The unit makes use of the IITC hardware system consisting of an automatic scanner pump, sensing tail cuff and amplifier that measures blood pressure in the animals ‘tail. The recorded

blood pressure of the tail vein of the animal is displayed on the computer screen. Prior to MAP measurement, the equipment was calibrated to eliminate any discrepancies. The animals were warmed in an enclosed chamber (IITC Life Sciences Inc., Woodland Hills, California, USA) for 30 minutes at ±30°C before taking three blood pressure recordings of each animal (Gondwe *et al.*, 2008).

Haematocrit levels were also monitored in all groups of animals at 0900 every 3rd day during the pre-treatment and post- treatment periods, and daily during the treatment period. Briefly, blood was collected into heparinised capillary tubes using the tail prick method. The sealed capillary tubes were then centrifuged for 3 minutes at 906 x G with a Micro-haematocrit centrifuge (346 MSE Centrifuge Manufacturer, London, UK). Haematocrit was determined by calculating the percentage of the total blood volume to packed cell volume.

2.8. Terminal studies

To assess the effects of CHQ treatments on some biochemical parameters, plasma CHQ profiles, electrolytes, AVP and insulin concentrations, separate groups of non-fasted conscious animals (n=6) were sacrificed during pre-treatment at days 0 and 7, treatment period at days 8, 9, 11, 12 and post-treatment period at day 21. The animals were anaesthetized by placing them in an anaesthetic chamber with 100 mg/kg of halothane for 3 minutes. Blood samples were collected by cardiac puncture into pre cooled heparinised tubes and centrifuged for 15 minutes at 959 x G at 4 °C (Eppendorf International, Hamburg, Germany). The plasma samples were stored in a Bio Ultra freezer (Snijers Scientific, Tilburg, Netherlands) at -80 °C until assayed. The collected plasma samples were used for electrolytes (Na^+, K^+, Cl^-), urea, creatinine, CHQ, insulin and AVP. Kidneys were collected, weighed gravimetrically (Mettler balance PC 180-insruments, Protea Laboratory Services, South Africa) and snap frozen in liquid nitrogen, to assess the effects of both infection and CHQ treatments on kidney weights.

2.9. Laboratory analysis

2.9.1. CHQ concentration measurements

Plasma CHQ concentrations were measured in separate groups of animals following treatment with either oral CHQ or pectin-CHQ matrix patch using a double extraction process as previously reported (Musabayane *et al.*, 2000) with slight modifications. Prior to use, all glassware was coated with sigmacote to prevent the adhesion of CHQ to the glass surfaces. Plasma sample (0.2 mL) was added to a 250 mL volumetric flask, followed by 1 mL of 25% potassium hydroxide and 10 mL of diethyl ether. The solution was mixed for 1 minute and allowed to stand for 2 minutes at room temperature. A 1% solution of sulphuric acid (2 mL) was subsequently added to the mixture for acidification. Absorbance of resultant solution was read spectrophotometrically (Ultraviolet Novaspec II spectrophotometer (Biochrom LTD, Cambridge, England) against a CHQ-free plasma blank at 343 nm. The obtained absorbance values were extrapolated from the standard curve to determine plasma CHQ concentrations Prior to plasma CHQ measurements, a CHQ standard curve was constructed using various CHQ concentrations ranging between 1.5 – 50.0 µg/mL. These CHQ concentrations were freshly prepared and read at 343 nm using Ultraviolet Novaspec II spectrophotometer (Biochrom LTD, Cambridge, England) and absorbance values were used to construct the standard curve, which was subsequently used to extrapolate plasma CHQ concentrations in test samples

2.9.2. Electrolytes measurements

Urinary and plasma Na^+, K^+, Cl^- and creatinine concentrations were determined by ion activity using the Beckman Coulter (Synchron LX20 Clinical Systems, Fullerton, Carlifornia, USA). Creatinine estimation employed the reaction of creatinine and picric acid at alkaline pH to form a yellow-orange complex, creatinine picrate. For the estimation of urea, the hydrolytic degradation of urea in the presence of the enzyme urease was used. Standard kits and reagents purchased from Beckman Coulter, Dublin, Ireland were used for the analysis. Glomerular filtration rate (GFR) assessed by creatinine clearance (C_{cr}) was calculated using a standard formula [C_{cr}=Urine creatinine concentration x Urine flow rate (V) / Plasma creatinine concentration] based on the measurements of the plasma and urinary concentrations of creatinine and urine flow rate.

2.9.3. Hormone analysis

2.9.3.1. Insulin assay

Plasma insulin concentrations were measured using the ultra-sensitive rat insulin ELISA kit (DRG diagnostics EIA-2943 GmbH, Marburg, Germany). The kit consisted of a 96 well plate coated with mouse monoclonal anti-insulin, enzyme conjugate, standards, enzyme conjugate buffer, substrate 3,3',5,5'-tetramethylbenzidine (TMB), wash buffer and a stop solution. This assay is a solid phase two-site immunoassay based on the direct sandwich technique in which two monoclonal antibodies are directed towards separate antigenic determinants on the insulin molecule. During the incubation period insulin in the sample reacts with peroxidase-conjugate anti-insulin antibodies bound to the microtitration well. The unbound enzyme labeled antibody was removed by the washing step, leaving the bound conjugate to react with TMB. The reaction was stopped by adding sulphuric acid to give a colorimetric endpoint which is read spectrophotometrically on a microplate reader (BMG Labtech GmbH, Ortenberg, Germany) at 450nm.

Each determination was done in duplicate for standards and test samples. A 50µl of each insulin standard was added to anti-insulin wells. Plasma samples (50µl) were added to the remaining anti-insulin wells, this was followed by the addition of the enzyme conjugate to standard and plasma samples wells. The plates were incubated at room temperature on a plate shaker (Heidolph, Schwabach, Germany) for 2 hours. This was followed by multiple wash using a wash buffer (350µl). After the final wash, the plates were inverted against absorbant paper to remove all the liquid in the plates. The substrate, TMB was then added to all wells and incubated for 30 minutes. The reaction was stopped by adding 50 µl of stop solution to all wells and mixing for 5 minutes. Absorbance was measured using Spectrostar Nano microplate reader (BMG Labtech GmbH, Ortenberg, Germany). A standard curve was constructed from the absorbance values of the known concentrations of the standards using the spline regression curve. Insulin concentrations of the unknown samples were extrapolated from the standard curve. The lower and upper limits of detection were 1.39 pmol/L and 960 pmol/L, 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%.

2.9.3.2. Arginine vasopressin (AVP) assay

A **s**tandard enzymatic method was used to determine plasma AVP concentrations. The assays were performed on an Arg^8-Vasopressin ELISA Kit, using reagents purchased from the manufacturer (Abcam, Cambridge, Massachusetts, USA). The lower and upper limits of detection were 4 pmol/L - 923 pmol/L, respectively. The intra assay analytical coefficient of variation ranged from 5.9 to 10.6% and the inter-assay coefficient variation from 6.0 to 8.5%.

2.9.3.2.1. Principle of the assay

The kit components included a 96 well plate coated goat antibodies , vasopressin conjugate, vasopressin antibody, assay buffer, wash buffer concentrate, vasopressin standards, p-nitrophenyl phosphatesubstrate (pNpp) and a stop solution. The Arg8-Vasopressin ELISA Kit is a competitive immunoassay for the quantitative determination of vasopressin in samples. The assay uses a polyclonal antibody-vasopressin conjugate to bind covalently in a competitive manner with vasopressin in unknown samples. During the incubation period AVP in the sample reacts with phosphatase-conjugate anti-vasopressin antibodies and anti-vasopressin antibodies bound to the microtitration well. The washing step removes unbound enzyme labeled antibody, leaving the bound conjugate which reacts with pNpp. This reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically on a microplate reader (BMG Labtech GmbH, Ortenberg, Germany) at 405 nm.

2.9.3.2.2. AVP extraction

Plasma samples were subjected to an extraction process prior to use. Briefly, 100 µL of plasma sample and 200 µL of ice cold acetone were added into the eppendorf tubes. The mixture was vortex and centrifuged at 12000 x G for 20 minutes. After centrifugation, the supernatant was collected into a glass tube. A volume of 500 µL of ice cold petroleum ether was added to the supernatant. The mixture was centrifuged at 10000 x G for 10 minutes to obtain the ether and aqueous layer. The remaining aqueous layer was transferred into new glass test tubes and dried under gas for 48 hours. After the drying, white crystals were obtained and were reconstituted in assay buffer. The samples were used immediately.

2.9.3.2.3. Assay procedure

Each determination was performed in duplicate for both standards and the test samples. The assay procedure was as follows: A volume of 100 µL of vasopressin standards (4, 10, 23, 59, 148, 369 and 923 pmol/L) was added into anti-vasopressin wells. Samples (100 µL) were then added to the remaining wells followed by 50 µL vasopressin conjugate into all standard and sample wells. The plates were incubated at 4ºC for 24 hours. Following incubation, the reaction volume was emptied. 400µL of wash buffer was added to all wells and aspirated. The process was repeated 3 times. After the final wash, the plates were inverted firmly against absorbent paper to remove all the liquid. 200µL of substrate pNpp was added to all wells and incubated at 37ºC for 1 hour. The reaction was stopped by adding 50µL of stop solution to all wells and mixing on the shaker for 5 minutes. The absorbance was read at 405 nm using a Spectrostar Nano microplate reader (BMG Labtech GmbH, Ortenberg, Germany). A standard curve was constructed from the absorbance values of the known concentrations of the standards using the spline regression curve in Graph Pad Instat software (version 5.00). The respective AVP concentrations of the unknown samples were then extrapolated from the standard curve.

2.10 Data analysis

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. For chronic studies, the calculation of mean daily fluid voided and urinary amounts of electrolytes excreted, plasma insulin and AVP concentrations were used to assess renal function. GFR was evaluated by creatinine clearance as assessed by 24-hour urinary excretion rates of creatinine inrelation to plasma concentration. The MAP was presented graphically for the separate groups of animals. GraphPad InStat Software (version 5.00, GraphPad Software, San Diego, California, USA) was used for all statistical analyses. One way analysis of variance (ANOVA) followed by Tukey-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

RESULTS

3.0. General

This chapter describes the following results:

1) Percentage CHQ incorporation of the pectin patch

2) Effects of oral and transdermal CHQ treatments on:
   i. Parasitaemia
   ii. Body weight, food and water intake
   iii. Glucose homeostasis
   iv. Renal function and
   v. Blood pressure

3) CHQ pharmacokinetics

3.1. CHQ incorporation in the pectin patch

To determine the amount of CHQ loaded in the pectin hydrogel matrix formulation, dissolution studies were conducted on freshly prepared CHQ patches. The theoretical amounts of CHQ in the patches calculated based on the amount of CHQ added initially during patch preparation was considered 100%.

Table 1: The total CHQ amounts in pectin CHQ patches with their corresponding percentage loading efficiencies (n=6 in each group)

Area (mm^2)	Theoretical CHQ concentration in patch (mg/mL)	CHQ concentration in patch (mg/mL)	Percentage CHQ incorporation (%)
4 ± 1	17	9	52
4 ± 1	22	16	74

3.2. Short-term studies

The effects of twice daily oral administration of CHQ (30 mg) for 5 consecutive days and a once-off topically applied pectin-CHQ hydrogel formulation (56 mg) on parasitaemia, physical parameters, glucose homeostasis, renal function and blood pressure were evaluated in separate groups of non-infected and *P.berghei*-infected rats treated with a once-off topical application of the pectin-CHQ matrix patch.

3.2.1. Effects on parasitaemia

To assess the ability of the CHQ formulation to clear malaria parasites, the effects of a once-off topical application of the pectin-CHQ patch (56 mg) and oral CHQ (30 mg) treatments on *P. berghei*-infected rats for 3 weeks were compared

Following infection with *P.berghei*, percentage parasitaemia gradually increased reaching a peak of 55 ± 8% on day 12. Due to ethical reasons, the control animals were sacrificed on day 12 of the experimental period. As such, all the subsequent results showing the infected control animals will no post treatment period (days 13-21).

Oral CHQ treatment cleared the malaria parasites by day 7 as was indicated by a decrease in parasite density. The effects of treatment with the CHQ patch on blood parasite density in *P.berghei*-infected rats are shown in Figure 3; all residual parasites were cleared from the systemic circulation during the post-treatment period. Similar results were obtained in animals treated twice daily with oral CHQ.

Figure 4 shows the effects of CHQ treatments on *P. berghei*-infected rats. Animals treated with oral CHQ twice daily for 5 consecutive days showed a significant reduction in percentage parasitaemia by day 12 from 29.33 ± 1.43% to 1.02 ± 0.25%, while the once-off transdermal application of the CHQ patch (28 mg) reduced and cleared the malaria parasite by day 20 of the experimental period. Interestingly, the once-off topical application of the CHQ patch containing 56 mg CHQ significantly reduced the parasitaemia from 38.72 ± 1.25 % to undetectable levels by day 12 of the experimental period.

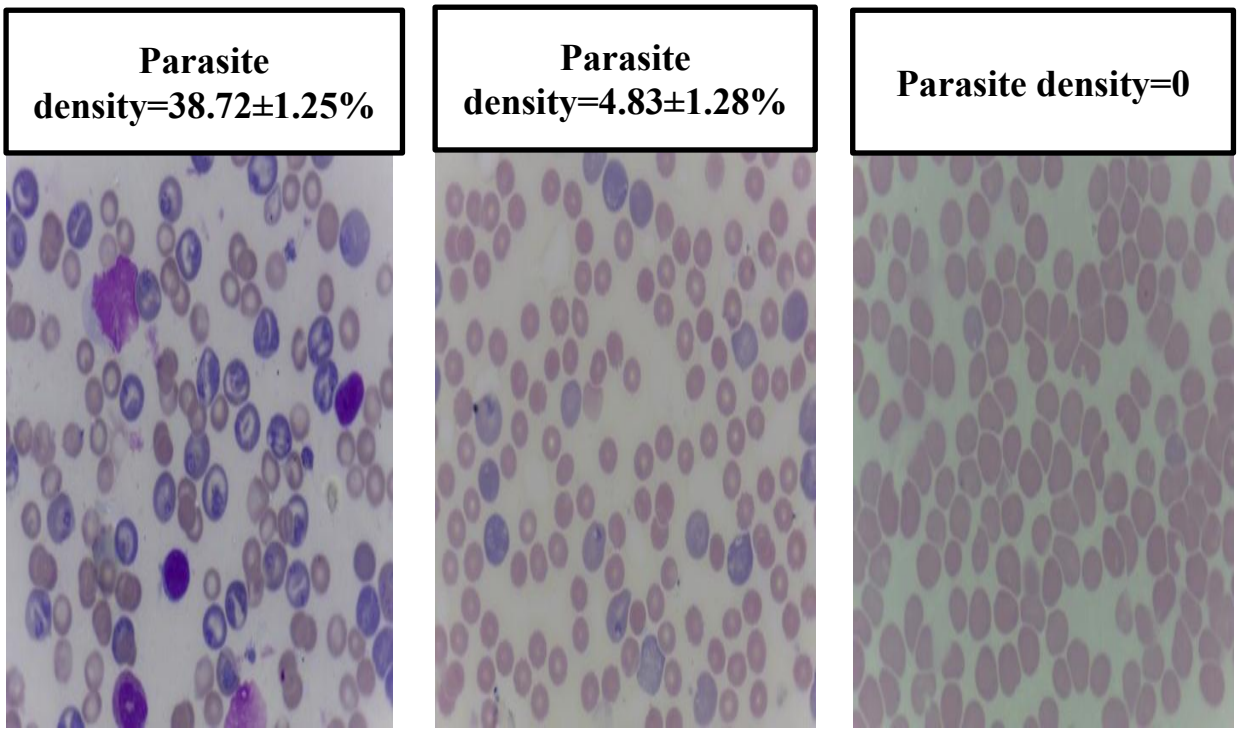

Figure 3: Parasite density in *P.berghei*-infected animals following a once-off topical application of the pectin-CHQ patch (56 mg) during the pre-treatment, treatment and post-treatment periods (Magnification, 100 x10). (Leica SCN 400, Leica Microsystems CMS GmbH, Watzlar, Germany)

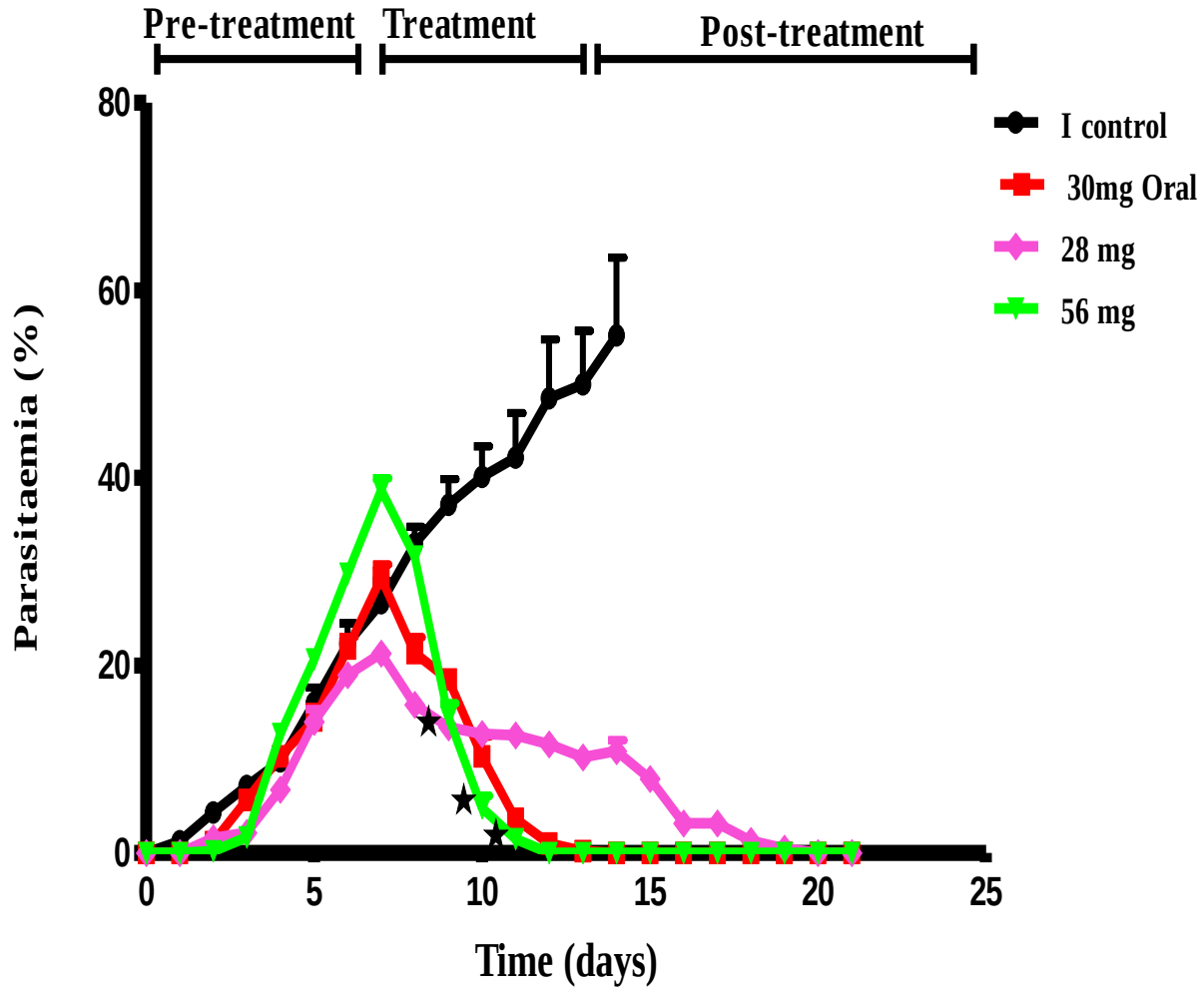

Figure 4: Comparison of the effects of twice daily oral CHQ (30 mg oral) and once-off patch (28 and 56) treatments on percentage parasitaemia in *P.berghei*-infected rats with untreated animals (I control). Values are presented as means and vertical bars indicate SEM (n=6 in each group) ★ $p<0.05$ by comparison to day 7**.**

3.2.2. Studies on physico-metabolic parameters

Body weight, food and water intake were monitored in non-infected and *P.berghei*-infected rats treated with either CHQ (30 mg, p.o) twice daily or a once-off topical application of the pectin-CHQ matrix patch (56 mg) to distinguish between the effects of oral and transdermal CHQ treatments on these parameters.

3.2.2.1. Body weight, food and water intake

Table 3 compares the effects of oral and transdermal CHQ treatments on mean body weight changes, food and water intake in separate groups of non-infected and *P. berghei*-infected rats. Untreated non-infected and *P. berghei*-infected rats served as non-infected (NIC) and infected controls (IC) respectively.

Untreated non-infected control animals progressively gained weight throughout the experimental period (Table 2). Oral administration of CHQ twice daily, for 5 consecutive days significantly decreased the mean body weight of non-infected rats by comparison with untreated non-infected rats. In addition to decreased mean body weight, the food and water intake was significantly reduced in these animals. Interestingly, non-infected rats treated with topically applied CHQ via the pectin-CHQ matrix patch progressively gained weight exhibiting mean values comparable to those of untreated non-infected control animals. However, the food and water intake were not altered by the pectin-CHQ patch application (Table 2).

The mean body weight of untreated *P. berghei*-infected rats significantly decreased by comparison with non-infected control rats throughout the 3-week study (Table 2). *P. berghei*-infected rats treated with oral CHQ twice daily exhibited significantly decreased mean body weights. In addition to the reduction in mean body weights, *P.berghei*-infected rats presented significantly ($p<0.05$) reduced food and water intake during the treatment period. However, the once-off topical application of the pectin-CHQ matrix patch significantly ($p<0.05$) increased the mean body weight of *P. berghei*-infected rats to values comparable to those of untreated non-infected control rats (Table 2). Furthermore, the total food and water intake of these animals was not altered by topical application of the pectin-CHQ (Table 2).

Table 2: Comparison of the effects of twice daily oral CHQ (30 mg) treatment with a once-off topically application of the pectin CHQ matrix patch (56 mg) on body weight changes food and water intake in non-infected and *P. berghei*- infected animals. Values are presented as means ± SEM, where columns represent means (n=6 in each group)

Parameter	Protocol		Baseline	Treatment	Post-treatment
Food intake (g/100g)	Control	Non-infected	9 ± 1	10 ± 4	9 ± 1
		P. berghei - infected	10 ± 1	7 ± 2 ★	N/A
	Oral	Non-infected	9 ± 1	7 ± 1★	9± 2
		P. berghei - infected	8 ± 1	6 ± 1★	8 ± 3
	Patch	Non-infected	10 ± 1	9 ± 1#	9 ± 1
		P. berghei - infected	9 ± 1	8 ± 2#	9 ± 2
Water intake (mL/100g)	Control	Non-infected	15± 2	13 ± 1	15 ± 2
		P. berghei - infected	8 ± 3	10 ± 2★	N/A
	Oral	Non-infected	10 ± 1	10 ± 1★	14 ± 1
		P. berghei - infected	8 ± 2	7± 2★	12 ± 2
	Patch	Non-infected	15 ± 1	14 ± 1#	14 ± 3
		P. berghei - infected	13 ± 1	13 ± 2#	15 ± 2
% b.wt change	Control	Non-infected	8±1	13 ±1★	18 ±1★
		P. berghei- infected	-6±2	-4±2★	N/A
	Oral	Non-infected	7±1	-2 ± 1★	8 ± 1
		P. berghei infected	-8±1	-7±1★	-1± 1★
	Patch	Non- infected	8±1	11+1★#	18±1★#
		P. berghei- infected	-6±1	-1±1★#	3±1★#

★$p<0.05$ by comparison to the non-infected control. # $p<0.05$ by comparison with oral CHQ treated animals

3.2.2.2. Effects on blood glucose concentrations

Blood glucose concentrations were monitored in separate groups non-infected and *P.berghei*-infected rats treated with either CHQ (30 mg, p.o) twice daily or a once-off topical application of the pectin-CHQ matrix patch (56 mg) to distinguish between the effects of oral and transdermal CHQ treatments.

The blood glucose concentrations of untreated non-infected control animals ranged from 5.95 ± 0.17 to 6.80 ± 0.24 mmol/L throughout the experimental period. Oral administration of CHQ to non-infected animals significantly ($p<0.05$) decreased blood glucose concentrations to a value of 3.03 ± 0.19 mmol/L (Figure 5). Interestingly, the once-off topical application of the pectin-CHQ patch did not alter the blood glucose concentrations of non-infected animals by comparison with untreated non-infected animals (Figure 6).

Untreated *P. berghei*-infected rats exhibited significantly reduced blood glucose concentrations by comparison to non-infected control animals. The administration of CHQ orally further reduced blood glucose concentrations of *P. berghei*-infected rats from day 8 to 12 of the experimental period by comparison to untreated infected control at the corresponding time period (Figure 5). Interestingly, the once-off topical application of the pectin-CHQ matrix patch significantly ($p<0.05$) increased blood glucose concentrations of *P. berghei*-infected animals from 9 to day12 reaching a mean value of 6.07 ± 0.14 mmol/L by day 12 of the experimental period (Figure 6).

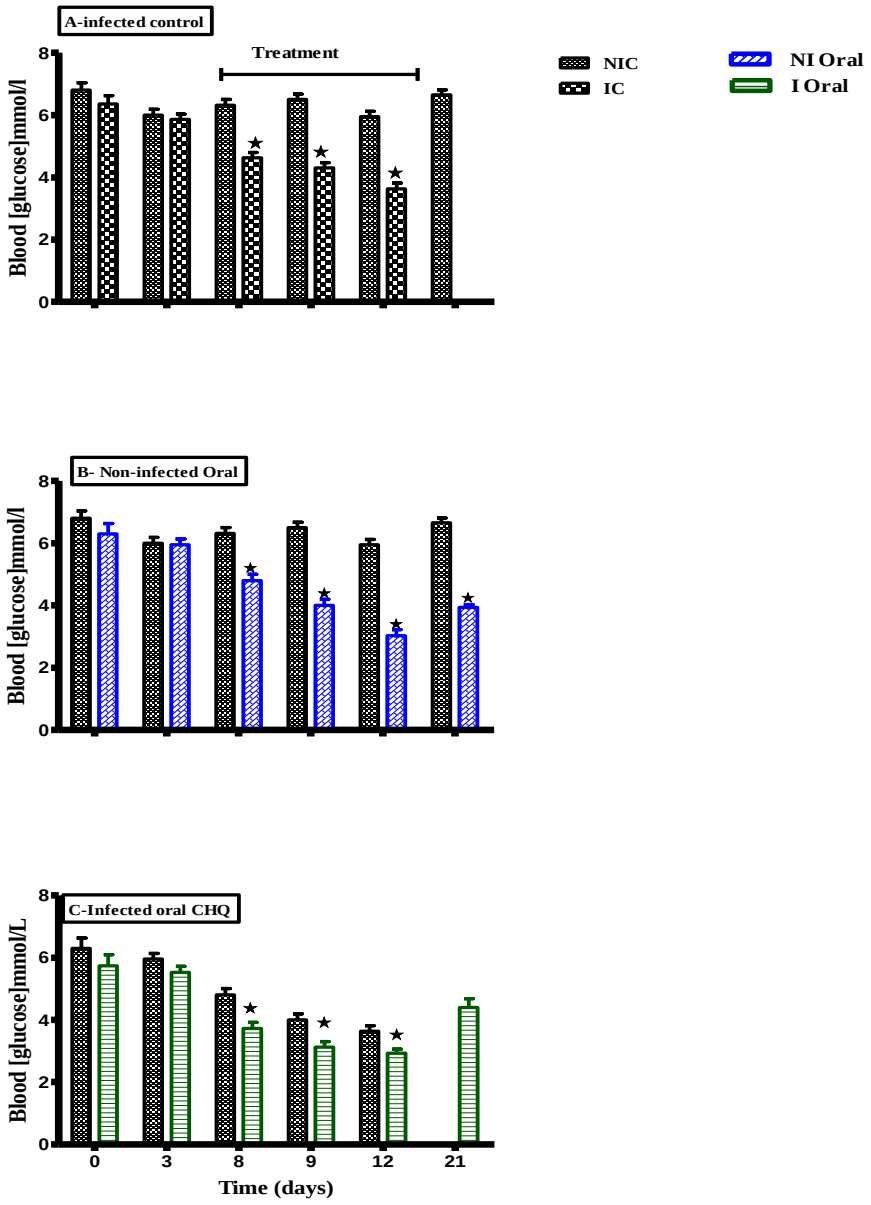

Figure 5: Blood glucose concentrations profiles of untreated non-infected **(A)**, oral CHQ (30 mg, p.o) treated non-infected **(B) (NI Oral)** and *P. berghei*-infected rats **(C) (I Oral).** Values are presented as mean ± S.E.M, where columns represent means and vertical bars represent SEM (n=6 in each group). ★$p<0.05$ by comparison to non-infected control (NIC) and infected control (IC) animals

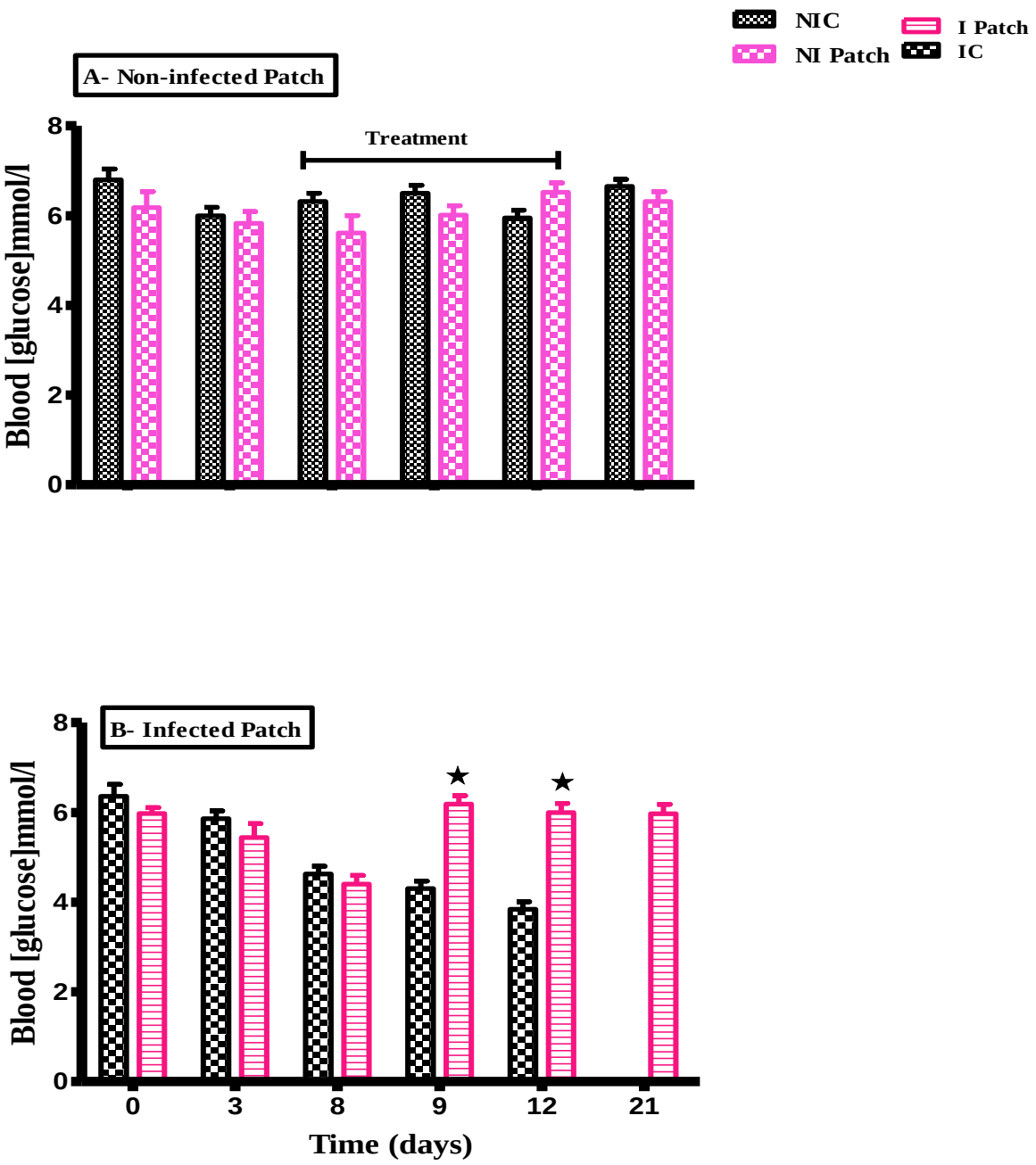

Figure 6: Blood glucose concentration profiles of non-infected **(A)** and *P. berghei* infected rats **(B)** treated with a pectin-CHQ matrix patch. Values are presented as mean ± S.E.M, where columns represent means and vertical bars represent SEM (n=6 in each group). ★$p<0.05$ by comparison to non-infected control (NIC) animals

3.2.2.3. Effects on plasma insulin concentrations

To elucidate the mechanisms responsible for the CHQ-induced hypoglycaemia, blood was collected from separate groups of non-infected and *P.berghei*-infected rats treated with either CHQ (30 mg, p.o) twice daily or a once-off topical application of the pectin-CHQ matrix patch (56 mg). Plasma insulin concentrations were measured from samples collected on day 7 of the pre-treatment period and day 8, 12 of the treatment period.

The plasma insulin concentrations of untreated non-infected rats remained unaltered throughout the study. Both oral CHQ administration and a once-off topical application of the pectin-CHQ patch significantly ($p<0.05$) increased the plasma insulin concentrations of non-infected animals by comparison with respective baseline values (Table 3).

There were no changes in plasma insulin concentrations of untreated *P.berghei*-infected control animals throughout the 3-week study. During the treatment (day 8-12) and post-treatment (day 21) periods, oral CHQ significantly ($p<0.05$) increased the plasma insulin concentrations of *P.berghei*-infected by comparison with baseline. Interestingly, following a once-off topical application of the pectin-CHQ patch, plasma insulin concentrations of *P. berghei* infected rats remained unchanged (Table 3).

Table 3: Comparison of CHQ, insulin and blood glucose concentrations in *P. berghei*-infected rats treated with either twice daily oral CHQ (30 mg) or a once-off topical application of the pectin CHQ matrix patch (56 mg) (n=6 in each group). Values are presented as means ± SEM

Treatment			Time (days)	[CHQ] µg/mL	Insulin pmol/L	Blood glucose mmol/L
Oral CHQ	Non-infected	Baseline	7	N/A	10 ± 1	6.41 ± 0.28
		Treatment	8	583 ± 24	28 ± 7★	5.98 ± 0.25
			12	289 ± 28	39 ± 4★	3.03 ± 0.19★
		Post-treatment	21	243± 59	13 ± 2	5.32 ± 0.13
	Infected	Baseline	7	N/A	11 ± 3	5.73 ± 0.36
		Treatment	8	574 ± 47	39 ± 4★	3.72 ± 0.20★
			12	386 ± 32	29 ± 7★	2.29 ± 0.13★
		Post-treatment	21	188± 18	16 ± 4★	4.40 ± 0.28★
Patch CHQ	Non-infected	Baseline	7	N/A	10 ± 1	6.41 ± 0.16
		Treatment	8	468 ± 18	20 ± 5#	5.62 ± 0.34#
			12	375 ± 20	18 ± 7#	6.53 ± 0.21#
		Post-treatment	21	279 ± 12	12 ± 1★#	6.53 ± 0.21
	Infected	Baseline	7	N/A	11 ± 3	5.98 ± 0.13
		Treatment	8	455 ± 28#	21 ± 3★#	4.43 ± 0.31★#
			12	365 ± 23#	22 ± 4★#	6.12 ± 0.19#
		Post-treatment	21	298 ± 18#	23 ±6★#	6.23 ± 1.03

★$p<0.05$ by comparison to baseline. # $p<0.05$ by comparison with oral CHQ treated animals.

3.2.3. Renal function studies

3.2.3.1. Effects on fluid, electrolyte handling and MAP

3.2.3.1.1. Non-infected groups

Figure 9 shows the urine volume voided, while Figure 7 shows urinary Na^+, K^+ and Cl^- outputs of control non-infected rats throughout the 21 day experimental period. Oral administration of CHQ (30mg), twice daily significantly ($p<0.05$) increased the urinary Na^+ and K^+ outputs from day 8 to day 12 of the experimental period, but the urinary Cl^- output was not altered. Interestingly, the treatment of non-infected animals with a once-off topical application of the pectin-CHQ matrix patch (56 mg) had no significant effect on the 24 hour volume of urine voided, Na^+, K^+ and Cl^- (Figure 11)

The MAP and haematocrit of untreated non-infected rats did not change throughout the 3-week study (Figure 9). However, oral administration of CHQ significantly ($p<0.05$) reduced both the MAP and haematocrit values of non-infected rats from 8 to day 12 by comparison with the untreated non-infected control animals (Figure 9). Interestingly, a once-off topical application of the pectin-CHQ matrix patch had no significant effect on the MAP and haematocrit values of non-infected animals (Figure 13)

3.2.3.1.1. *P. berghei*-infected groups

The mean daily urine voided, Na^+ and Cl^- outputs of untreated *P. berghei*-infected rats remained unchanged throughout the study. However, a significant increase in K^+ output was observed in these animals from day 8 to day 12 of the experimental period. Oral CHQ further increased urinary Na^+ and K^+ outputs at corresponding time periods (Figure 8), but the urine volume voided was reduced (Figure 10). Following a once-off topical application of the pectin-CHQ patch the mean K^+ output of *P. berghei*-infected rats was comparable to that of the untreated infected control animals, although the mean daily urine volume (Figure 12), urinary Na^+ and Cl were not altered.

Following infection with *P. berghei*, a significant ($p<0.05$) reduction in MAP and haematocrit values was observed in untreated control animals from day 3 to day 12 of the experimental period. This reduction in MAP and haematocrit values was exacerbated oral administration of CHQ, which significantly reduced both MAP and haematocrit from day 8 to day 12 by comparison with untreated infected control animals (Figure 10). Interestingly the MAP of *P. berghei*-infected animals treated with a once-off topically applied CHQ patch was not altered throughout the experimental period. However, a significant increase in haematocrit values was observed following treatment with the pectin-CHQ matrix patch from day 9 to day 21 of the experimental period (Figure 14).

In summary, oral administration of CHQ significantly reduced the MAP and haematocrit levels of non-infected and *P. berghei*-infected rats, while the pectin-CHQ patch maintains normal MAP and haematocrit values in *P. berghei*-infected animals. The mean urine volume, Na^+, K^+, Cl^- excreted and GFR during the treatment period are summarized in table 4.

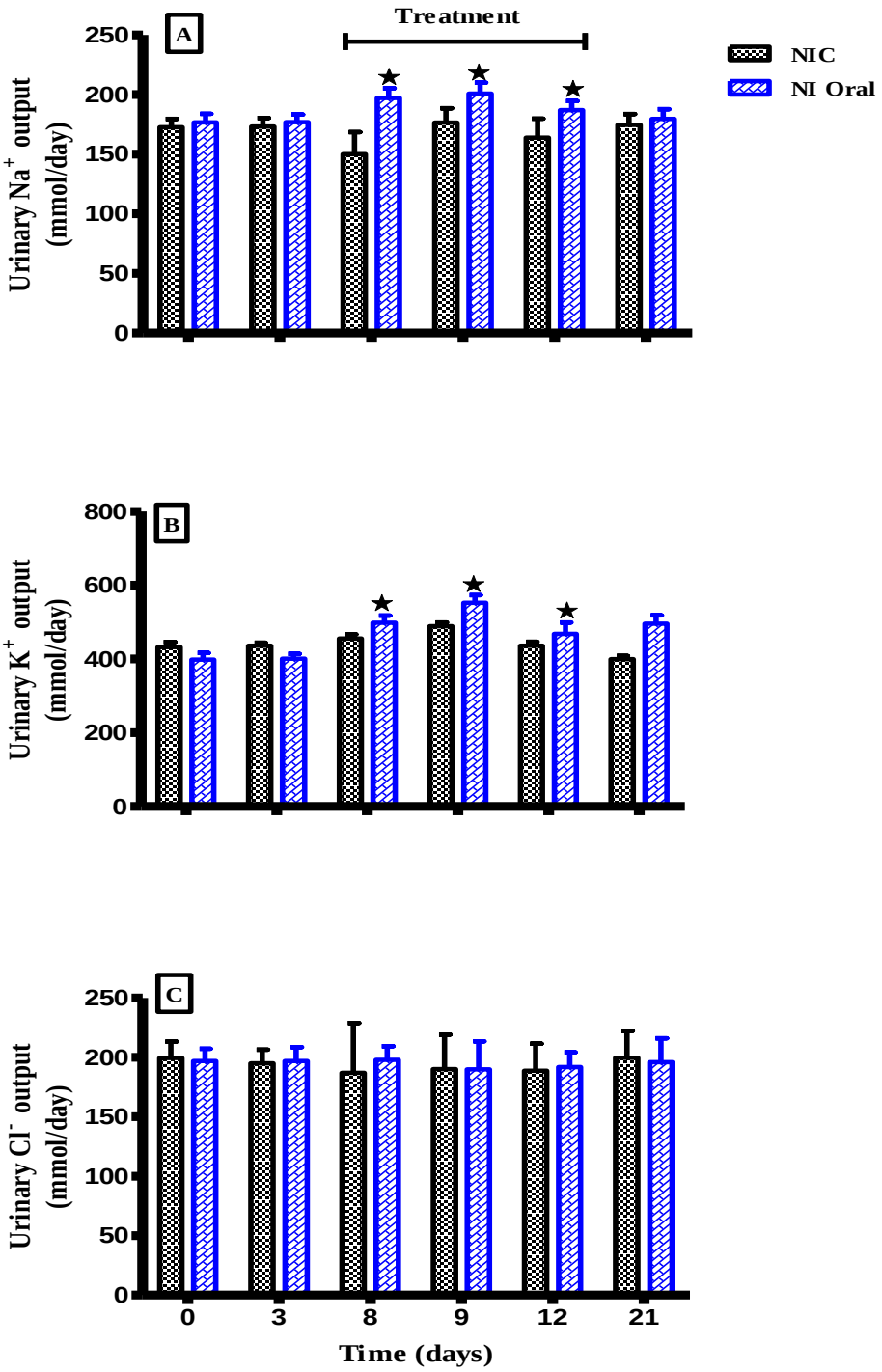

Figure 7: Comparison of the 24 hour urinary Na^+ **(A)**, K^+ **(B)** and Cl^- **(C)** outputs in untreated non-infected (NIC) rats with oral CHQ (NI Oral) treated rats. Values are presented as mean ±SEM, where columns represent means (n=6 in each group). ★$p<0.05$ by comparison to non-infected control (NIC) animals.

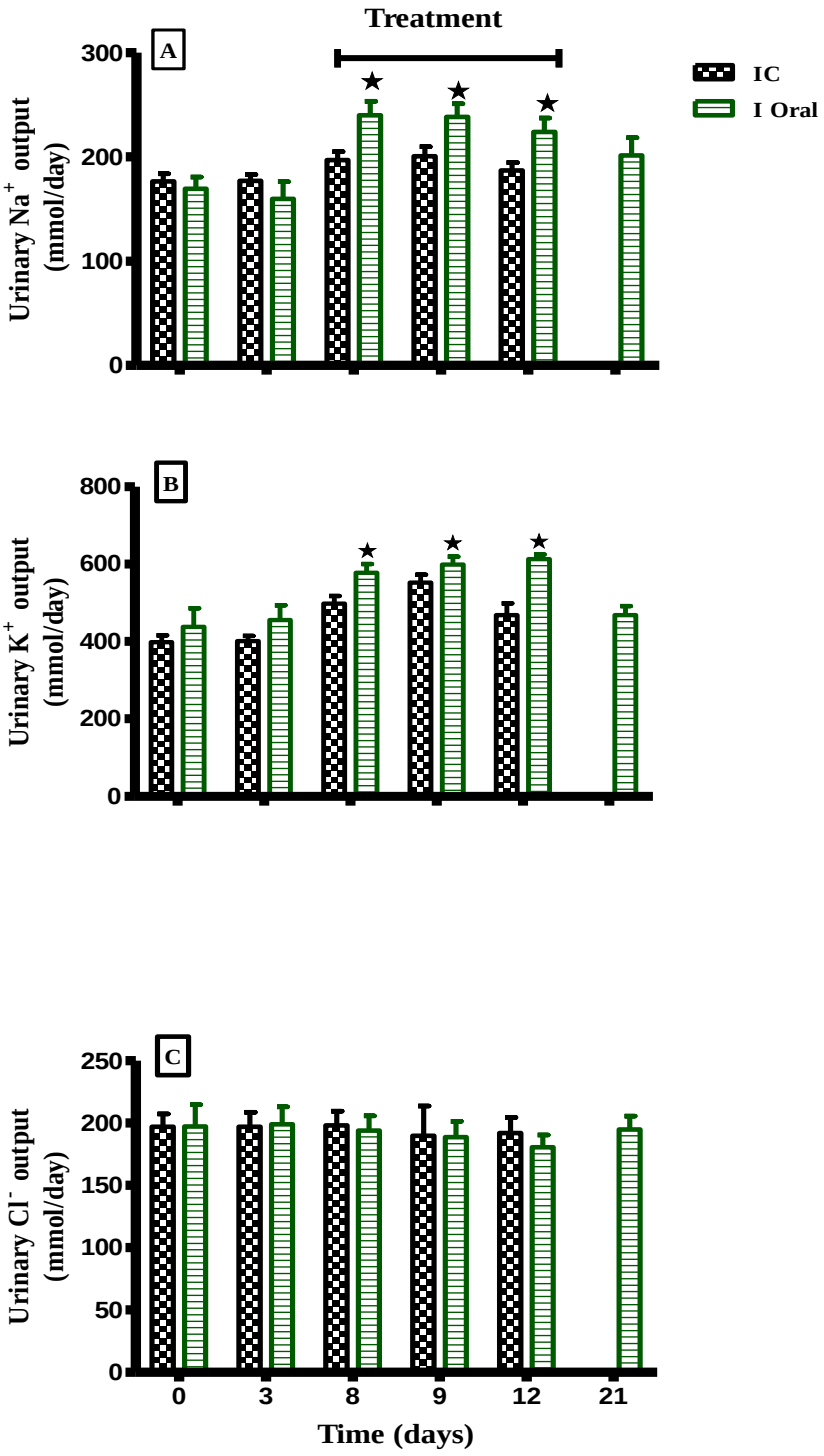

Figure 8: Comparison of the 24 hour urinary Na^+ **(A)**, K^+ **(B)** and Cl^- **(C)** outputs in untreated *P. berghei* infected (IC) rats with oral CHQ (I Oral) treated rats. Values are presented as mean ± SEM, where columns represent means (n=6 in each group). ★$p<0.05$ by comparison to non-infected control (IC) animals.

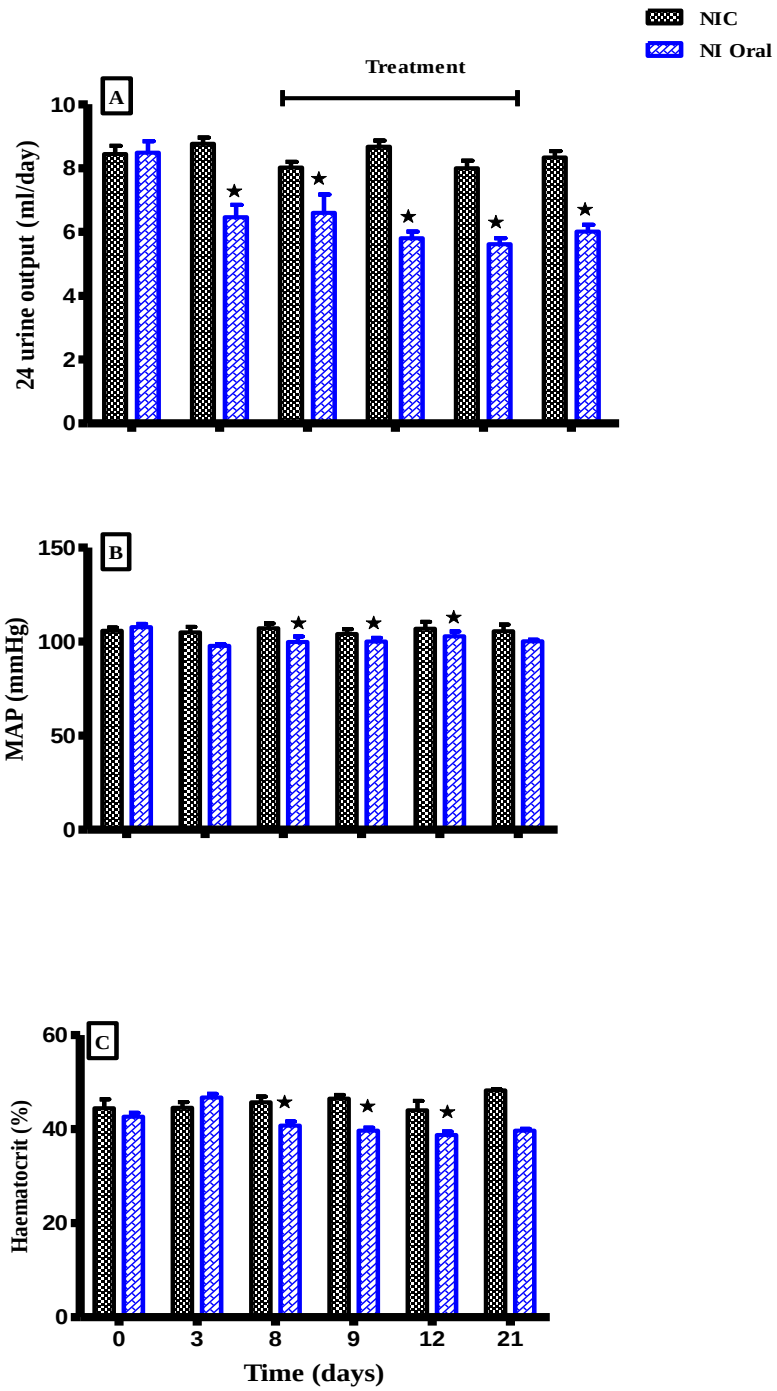

Figure 9: Comparison of the 24 hour urine output **(A)**, MAP **(B)** and haematocrit **(C)** in untreated non-infected (NIC) rats with oral CHQ (NI Oral) treated rats. Values are presented as mean ± SEM, where columns represent means (n=6 in each group). ★$p<0.05$ by comparison to non-infected control (NIC) animals.

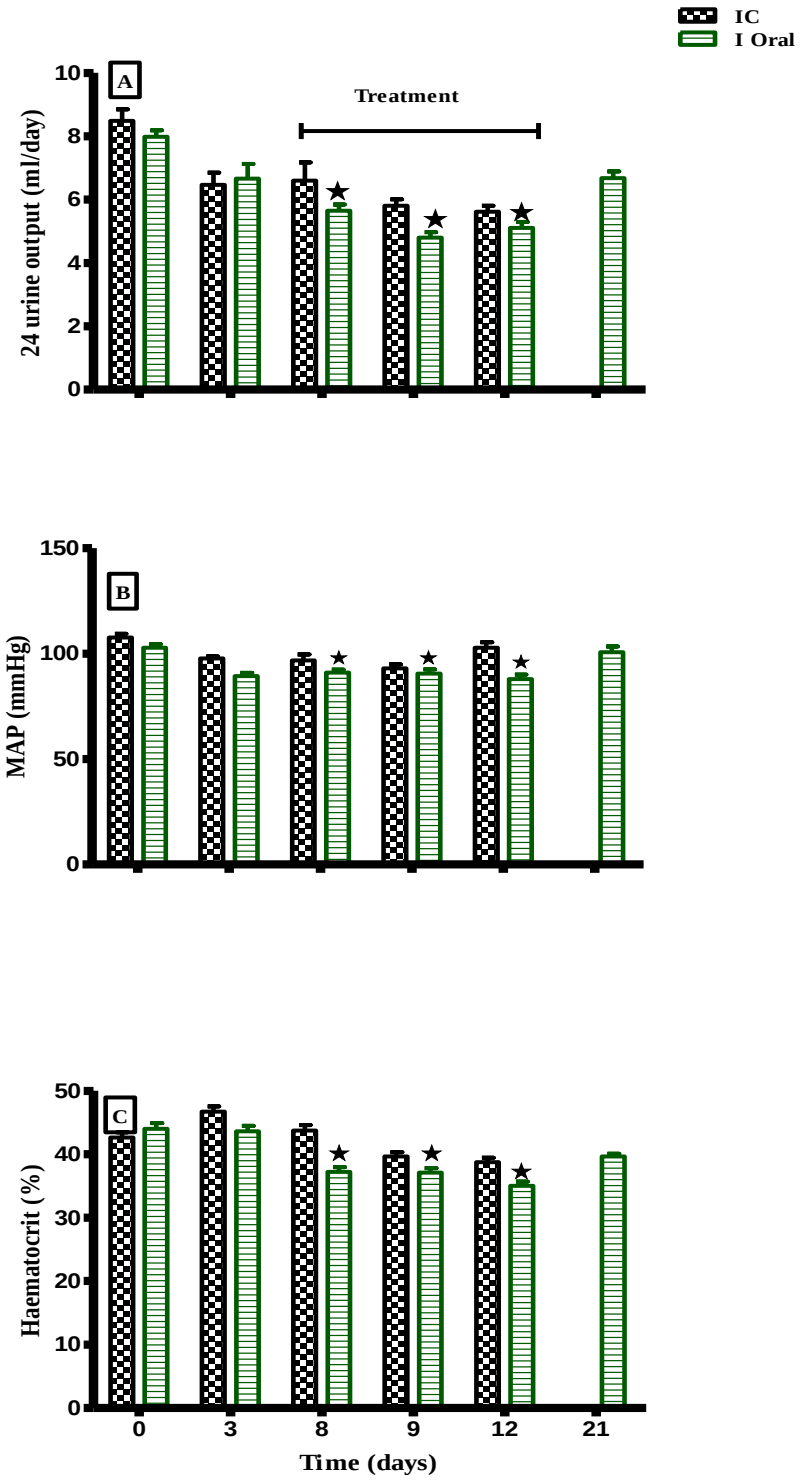

Figure 10: Comparison of the 24 hour urine output **(A)**, MAP **(B)** and haematocrit (**C)** in untreated *P. berghei*-infected (IC) rats with oral CHQ (I Oral) treated rats. Values are presented as mean ± SEM, where columns represent means (n=6 in each group). ★$p<0.05$ by comparison to non-infected control (IC) animals.

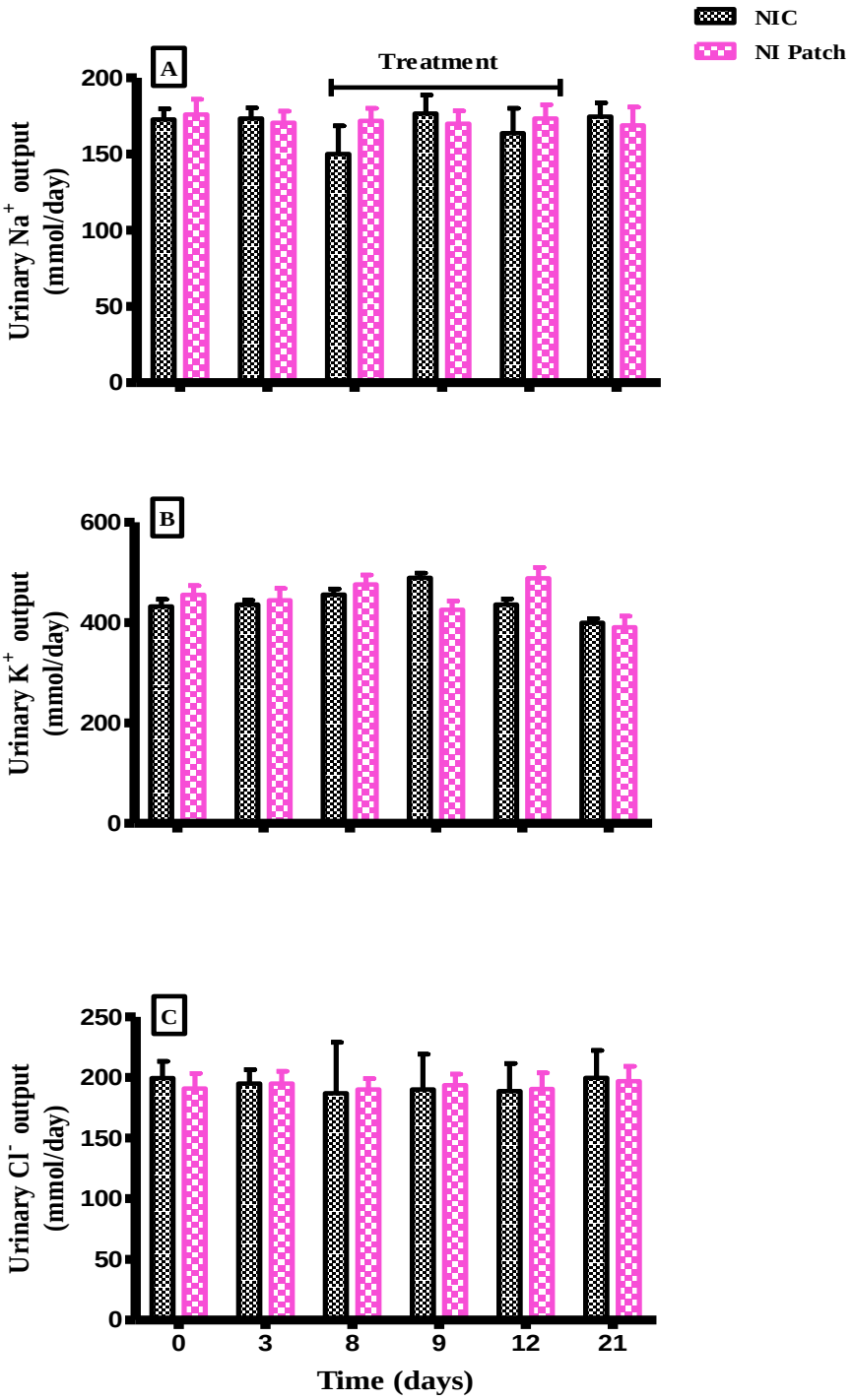

Figure 11: Comparison of the 24 hour urinary Na^+ **(A)**, K^+ **(B)** and Cl^- **(C)** outputs in untreated non-infected (NIC) rats with pectin-CHQ patch (NI Patch) treated rats. Values are presented as mean ± SEM, where columns represent means (n=6 in each group). ★$p<0.05$ by comparison to non-infected control (NIC) animals.

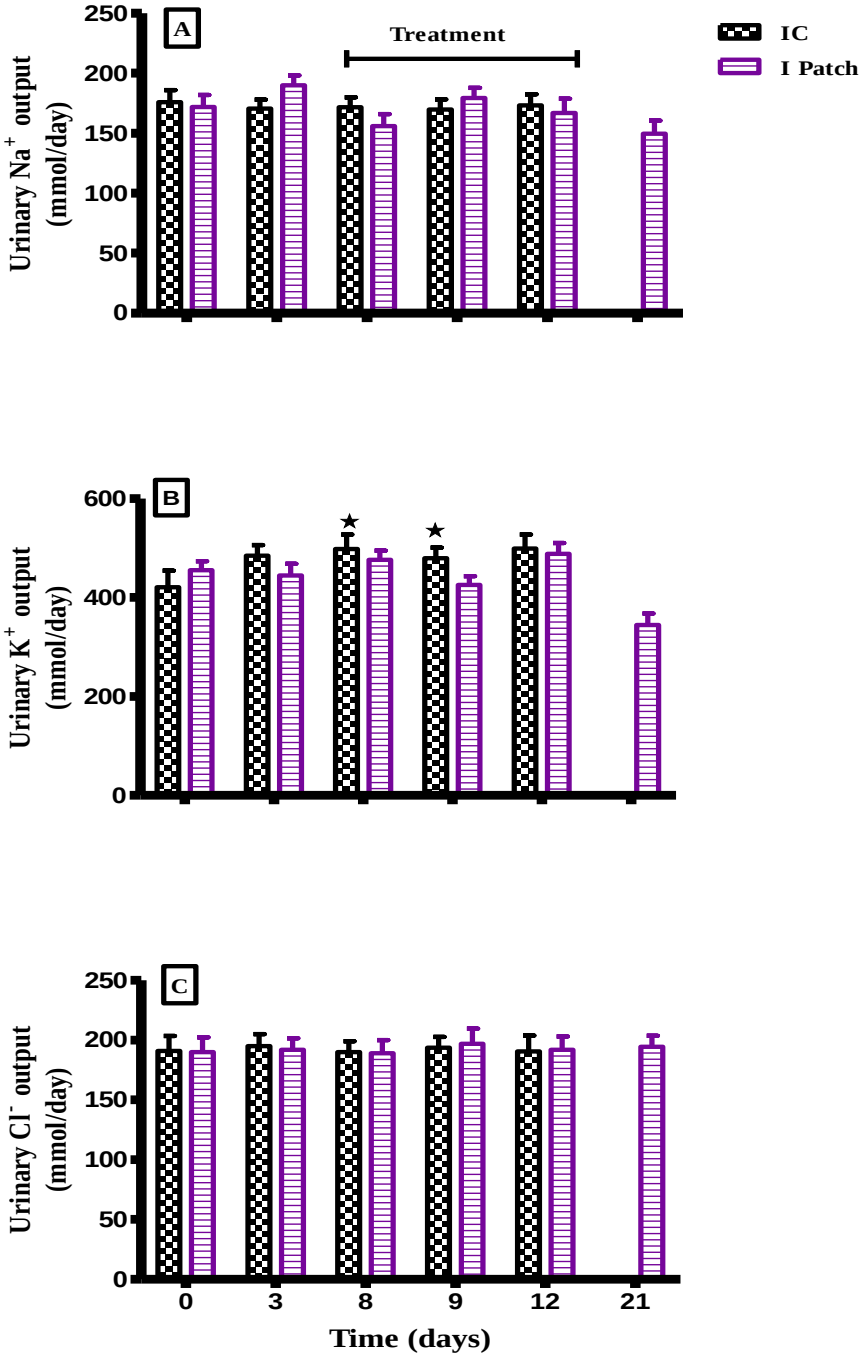

Figure 12: Comparison of the 24 hour urinary Na^+ **(A),** K^+ **(B)** and Cl^- **(C)** outputs in untreated *P. berghei*-infected (IC) rats with pectin-CHQ patch (I Patch) treated rats. Values are presented as mean ± SEM, where columns represent means (n=6 in each group). ★$p<0.05$ by comparison to non-infected control (IC) animals.

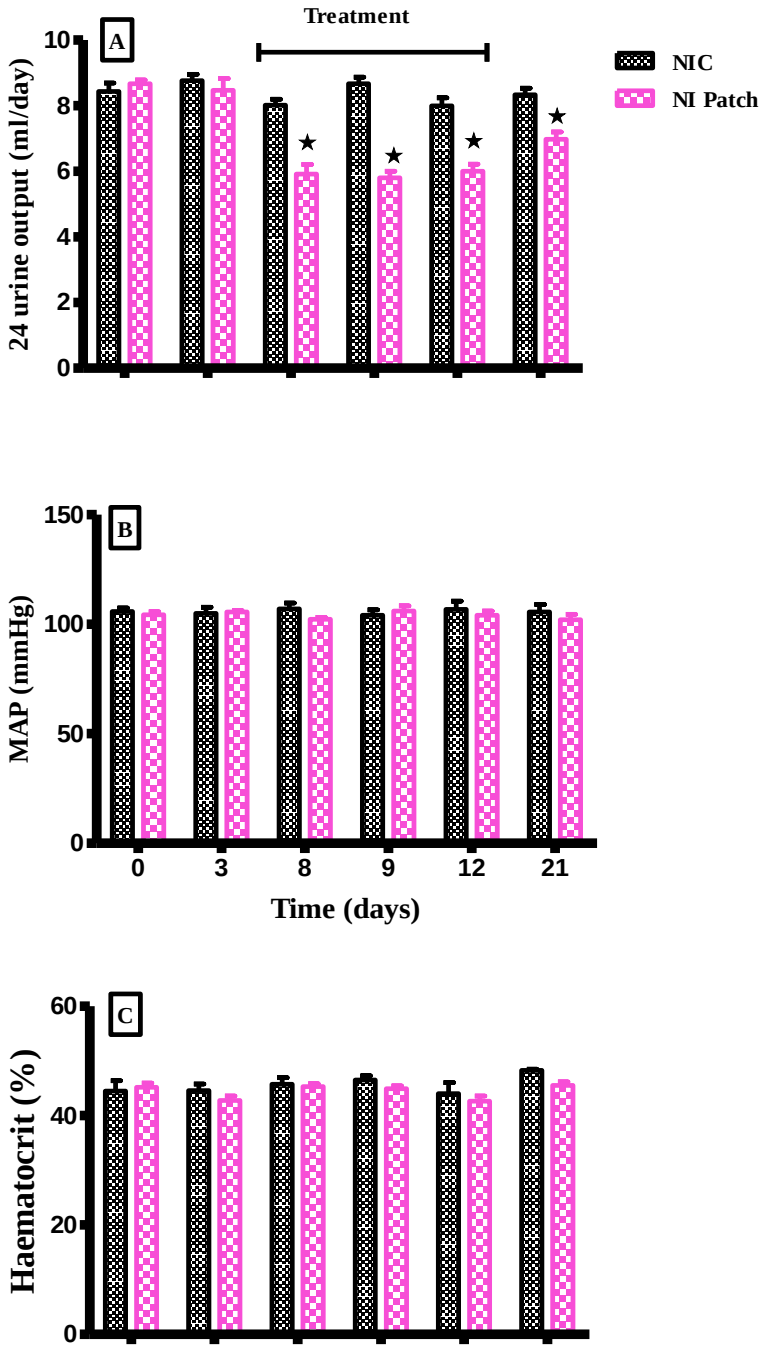

Figure 13: Comparison of the 24 hour urine output **(A),** MAP **(B)** and haematocrit **(C)** in untreated non-infected (NIC) rats with pectin-CHQ patch (NI Patch) treated rats. Values are presented as mean ± SEM, where columns represent means (n=6 in each group). ★$p<0.05$ by comparison to non-infected control (NIC) animals.

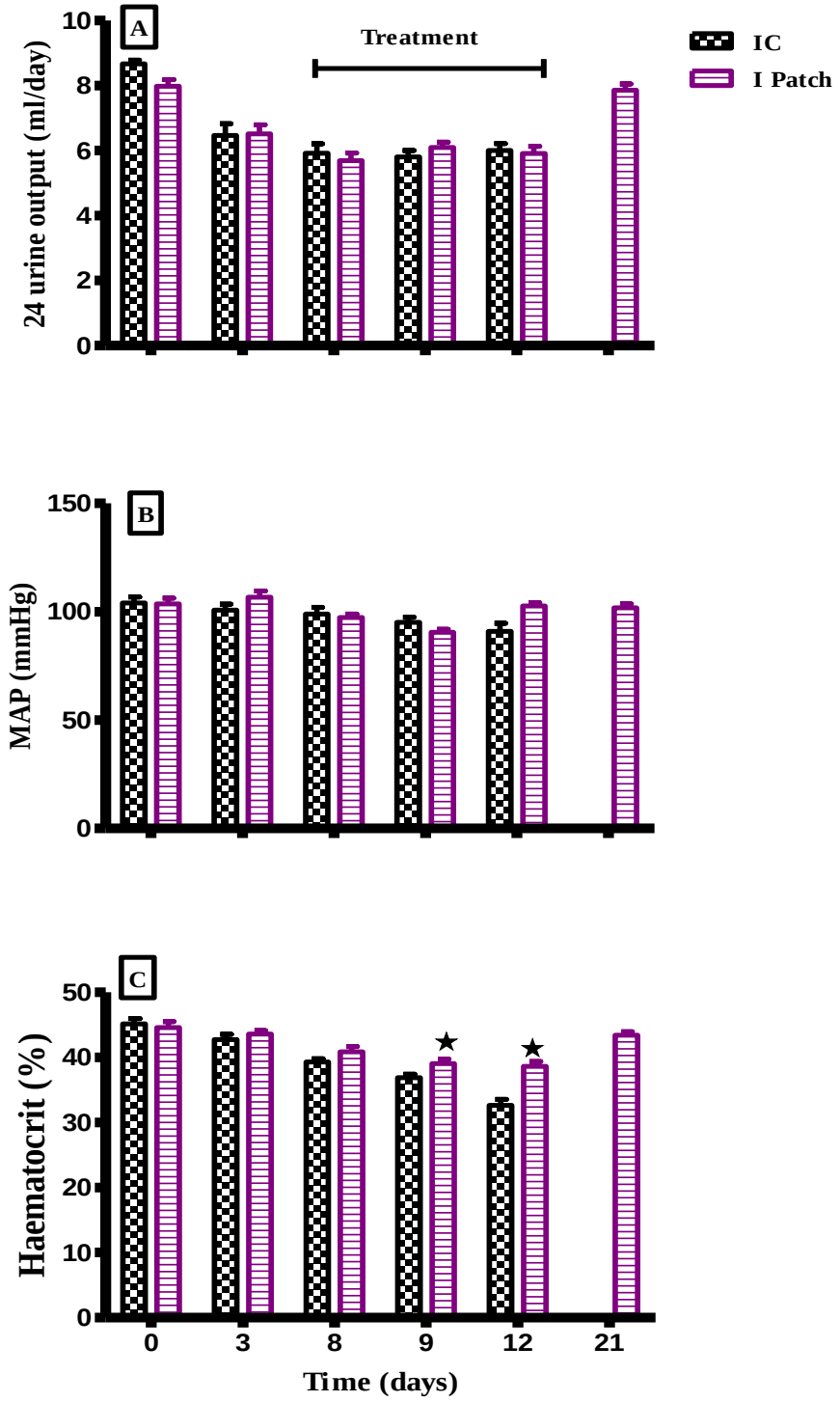

Figure 14: Comparison of the 24 hour urine output **(A),** MAP **(B)** and haematocrit **(C)** in untreated *P. berghei*-infected (IC) rats with pectin-CHQ patch (I Patch) treated rats. Values are presented as mean ± SEM, where columns represent means (n=6 in each group). ★$p<0.05$ by comparison to non-infected control (IC) animals.

3.2.3.2. Total urine volume, Na^+, K^+, and Cl^- excreted during the treatment period

The total amounts of fluid voided and electrolytes excreted in all groups during the treatment periods are summarized in Table 4.

Oral administration of CHQ significantly elevated Na^+ excretion in both non-infected and infected animals. In addition to natriuresis, the malaria parasite and oral CHQ induced hyperkalaemia in infected animals, while transdermal delivery of CHQ via the pectin-CHQ patch had no significant effects on renal fluid and electrolyte handling (Table 4).

Table 4: Comparison of total amounts of urine volume, Na+, K+ and Cl^- excreted during the treatment period by non-infected and *P.berghei*-infected rats treated with either oral or transdermally delivered CHQ with respective control animals (n = 6 in all groups). Data are expressed as means ± SEM (n = 6 in each group).

Parameter	**NIC**	**NI Oral**	**NI Patch**
Urine volume (mL)	8± 1	7 ± 1	6± 1
Na^+ (mmol/day)	18 ± 2	30 ± 3*	21 ± 2$^{\#}$
K^+(mmol/day)	56 ± 3	75 ± 3*	59 ± 3$^{\#}$
Cl^- (mmol/day)	23 ± 1	30 ± 2	25 ± 2
GFR (ml/min)	0.9± 0.2	0.8 ± 0.1	0.9 ± 0.1
	IC	**I Oral**	**I Patch**
Urine volume (mL)	6 ± 1$^{\infty}$	6 ± 1	6 ± 2
Na^+ (mmol/day)	26 ± 2$^{\infty}$	42 ± 3*	31 ± 2$^{\#}$
K^+(mmol/day)	101 ± 1$^{\infty}$	105 ± 2*	86 ± 3$^{\#}$
Cl^- (mmol/day)	34 ± 2	34 ± 2	33 ± 2
GFR (ml/min)	0.8 ± 0.3	0.9 ± 2	1.0 ± 0.2

$\star$ $p<0.05$ by comparison with the peak plasma CHQ concentration, # by comparison with oral CHQ treatment, ∞ by comparison with non-infected control.

Table 5: Plasma biochemical parameters of non-infected and *P.berghei*-infected rats treated twice daily with oral CHQ for 5 consecutive days or a once-off topical application of the pectin-CHQ matrix patch (n = 6 in all groups)

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)	134 ± 3	136 ± 4	140 ± 2	140 ± 41	138 ± 2	139 ± 4
	K^+ (mmol/l)	5.89 ± 0.80	6.70 ± 0.36	4.33 ± 0.82	7.09 ± 0.33★	5.36 ± 0.31	5.49 ± 0.44★#
	Cl^- (mmol/l)	102 ± 8	98 ± 8	102 ± 9	96 ± 7	103 ± 9	102 ± 9
	GFR (ml.min $100g^{-1}$)	0.80 ± 0.02	0.90 ± 0.01	0.90±0.02	0.90±0.02	0.80±0.01	1.10±0.02
	Kidney mass (g/100g b.wt)	0.80 ± 0.02	0.89 ± 0.01	0.89 ± 0.03	0.83 ± 0.01	0.80 ± 0.02	0.83 ± 0.02
Treatment	Na^+ (mmol/l)	131 ± 2	133 ± 4	134 ± 2	140 ± 4	139 ± 1	141 ± 2
	K^+ (mmol/l)	5.51 ± 0.30	4.50 ± 0.58	4.63 ± 0.40	10.50 ±1.56★	5.56 ± 0.31	5.49 ± 0.40#
	Cl^- (mmol/l)	104 ± 3	101 ± 3	103 ± 3	101 ± 2	98 ± 3	103 ± 3
	GFR (ml.min.$100g^{-1}$)	0.91±0.03	0.83±0.02	0.80±0.02	0.90±0.01	0.90± 0.03	0.89±0.03
	Kidney mass (g/100g b.wt)	0.90 ± 0.04	1.01 ± 0.40	0.89 ± 0.02	0.93 ± 0.04	0.90 ± 0.04	0.93 ± 0.05
Post-treatment	Na^+ (mmol/l)	130 ± 3	N/A	131 ± 3	137 ± 4	147 ± 1	139
	K^+ (mmol/l)	5.39 ± 0.10	N/A	3.73 ± 0.3	10.09± 4.06★	4.37±0.79	4.37 ± 0.80#
	Cl^- (mmol/l)	101 ± 1	N/A	101 ± 3	103 ± 2	102 ± 4	101 ± 2
	GFR(ml.min.$100g^{-1}$)	0.90±0.01	N/A	1.02±0.03	1.0±0.01	1.2±0.02	1.30±0.03
	Kidney mass (g/100g b.wt)	0.83 ± 0.03	N/A	0.90 ± 0.01	0.90 ± 0.03	0.88±0.02	1.03 ± 0.07

★ $p<0.05$ by comparison with the peak plasma CHQ concentration, # by comparison with oral CHQ treatment

3.3. Laboratory analysis

3.3.1. Plasma CHQ pharmacokinetics

Transiently high plasma CHQ concentrations have been previously reported following oral CHQ administration. Accordingly we evaluated and compared the effects of a once-off topical application of the pectin-CHQ patch (56 mg) treatment on plasma CHQ concentration to those of oral (30 mg, p.o) twice daily. Blood was collected from separate groups of *P. berghei*-infected rats treated with either oral or pectin-CHQ patch during the treatment (day 8-12) and post-treatment period (day 21).

Table 6 shows plasma CHQ concentration profiles at different time intervals following oral CHQ treatment, twice daily, 8 hours apart and a once-off transdermal application of the pectin matrix patch. Significantly ($p<0.05$) high plasma CHQ concentrations were observed on day 1 of the treatment period in both orally and transdermally treated animals. However these high plasma CHQ concentrations were significantly ($p<0.05$) reduced by day 2 in animals treated with oral CHQ. During the post treatment period, plasma CHQ concentrations were significantly reduced in the oral CHQ treated group by comparison to the patch treated group. . The once-off topical application of the pectin-CHQ matrix patch treatment sustained plasma CHQ concentrations for 5 days as shown in table 6.

Table 6: Plasma CHQ profiles of *P. berghei*-infected rats treated twice daily with oral CHQ (30mg) treatment and transdermally applied CHQ (56 mg). Values are presented as means ± SEM. (n=6 in each group)

	Time (Days)	**CHQ concentration (µg/mL)**	
		Oral CHQ	**Pectin-CHQ matrix patch**
Treatment	8	583 ± 24	455 ± 28
	9	396 ± 24★	410 ± 24
	11	487 ± 31★	399 ± 24 #
	12	289 ± 28★	365 ± 23★#
Post-treatment	21	188 ± 18★	298 ± 18★#

★ $p<0.05$ by comparison with the peak plasma CHQ concentration, # by comparison with oral CHQ treatment

3.3.2. Effects of CHQ treatments on plasma AVP

To elucidate the possible mechanism responsible for the natriuretic effects of oral CHQ, plasma AVP concentrations were measured on samples collected from *P. berghei*-infected rats treated with either oral CHQ or the pectin-CHQ matrix patch. The samples were collected on day 1, day 4 of the treatment and day 21 of the post-treatment period. Plasma samples that were collected on 7 of the pre-treatment period served as baseline.

Table 7 shows the effects of twice daily oral CHQ administration and a once-off topical application of the pectin-CHQ matrix patch on plasma AVP concentrations of *P.berghei* -infected rats during the treatment and post-treatment periods. Oral CHQ treatment significantly ($p < 0.05$) increased plasma AVP during the treatment period by comparison to baseline samples collected on days 7 from untreated *P. berghei*-infected rats. A once-off topical application of the pectin-CHQ matrix patch had no significant effects on plasma AVP concentrations by comparison to baseline.

Table 7: Comparison of plasma AVP concentrations in *P. berghei*-infected rats treated with either oral CHQ (30 mg) or CHQ patch (56 mg). Values are presented as means ± SEM, where columns represent means and vertical bars represent SEM (n=6 in each group)

	Plasma AVP (pmol/L)	
	Oral CHQ	Patch CHQ
Baseline	481 ± 62	481 ± 62
Treatment	584 ± 29★	496 ± 48#
Post-treatment	535 ± 36	508 ± 34

★ $p<0.05$ by comparison with baseline #$p<0.05$ by comparison with oral treated animals

CHAPTER 4

DISCUSSION

4.0. General

The present study was primarily designed to develop a novel CHQ-formulation that delivers sustained slow CHQ release into the systemic circulation. We also investigated the ability of this CHQ formulation to clear the malaria parasites in *Plasmodium berghei*-infected male Sprague-Dawley rats. The other objective of the study was to investigate and distinguish between the patho-physiological effects of malaria parasites and CHQ treatment on blood glucose homeostasis and renal fluid and electrolyte handling in rats. A rodent infecting, CHQ susceptible *P. berghei* was used for the induction of malaria. *P. berghei* widely used as an experimental model to study human infecting malaria is considered a safe model since this species of *Plasmodium* does not affect humans (Janse & Waters, 1995). Male Sprague Dawley rats (90-120) were used for the induction of malaria. These are growing rats and are highly susceptible to infection by the *Plasmodium* parasites. This makes these rats good experimental models since they are able to reach peak percentage parasitaemia which allowed for the investigation of the antimalarial activity of our novel CHQ-formulation. The results indicate that we were able develop a novel CHQ-formulation and achieved drug percentage incorporations ranging between 52-74%, these are the acceptable incorporations for transdermal drug delivery. Moreover, the mounts of CHQ obtained in the pectin-CHQ matrix patches equates to clinical doses. We have also demonstrated the ability of the pectin-CHQ matrix patch formulation to deliver sustained controlled therapeutic doses of CHQ which cleared the malaria parasites within a period of 5 days. Moreover the novel CHQ formulation not only clears the *Plasmodium* parasites, but sustains normal blood glucose concentrations and does not affect renal function of malaria infected animals. The current data suggests that transdermal CHQ delivery has the potential to ameliorate the pathophysiological effects that are associated with oral CHQ treatment and could provide an alternative method for the management of malaria.

4.1. Effects of CHQ treatments on parasitaemia

CHQ has been the mainstay therapy in the treatment of malaria in many tropics. The success of this anti-malarial drug is ascribed to the ability to inhibit the replication of the *Plasmodium* parasites, thus clearing the parasites from the systemic circulation (Zhang *et al.*, 1999). Oral administration of CHQ, twice daily, 8 hours apart for 5 consecutive days, and a once-off topical application of the pectin-CHQ matrix patch containing 56 mg CHQ reduced and cleared the malaria parasites. However, the pectin-CHQ matrix patch cleared the malaria parasites within a period of 5 days whereas the oral administration of CHQ only cleared the parasites after 7 days of treatment. The pectin-CHQ patch containing 28 mg CHQ, only cleared the *Plasmodium* parasites from the systemic circulation after 11 days of patch application when compared to orally administered CHQ which cleared the parasite after 7 days. This prolonged time period to clear the parasites by our pectin- CHQ matrix patch might be attributed to the fact that 28 mg CHQ was our lower CHQ dose.The ability of the once-off transdermal application of the pectin-CHQ patch to clear the malaria parasites within a period of five days provides for the first time, evidence of sustained, controlled release of CHQ from our transdermal formulation. The pectin-CHQ matrix patch was able to provide therapeutic concentrations at a sustained rate for up to 5 days. The current data suggests transdermal CHQ formulation as a practical alternative to conventional oral CHQ for malaria chemoprophylaxis and treatment.

4.2. Blood glucose homeostasis

To evaluate the effects of CHQ treatment on blood glucose homeostasis and to further distinguish between the effects of malaria parasites and CHQ treatment, *P. berghei*-infected rats were treated with either oral CHQ or a once-off topical application of the pectin-CHQ matrix patch. In addition the current study investigated the effects of a novel CHQ-formulation on blood glucose homeostasis in an effort to avert the adverse effects that are reported following oral administration of CHQ.

Following infection of rats with *P. berghei*, a significant reduction in blood glucose concentrations was observed. Previous studies have reported blood glucose lowering effects

by the malaria parasites which are mediated through various mechanisms (White *et al.*, 1987, Krishna *et al.*, 1999). These include the inhibition of gluconeogenesis and anaerobic glycolysis (White *et al.*, 1987, Taylor *et al.*, 1988). However, the utilization of the host's glucose stores by the malaria parasites is thought to be one of the main factors which precipitates hypoglycaemia in malaria patients (White *et al.*, 1983). The depletion of vital gluconeogenic substrates such as thiamine by the malaria parasite plays a major role in the reported malaria-associated hypoglycaemia (Krishna *et al.*, 1999). In addition, the malaria parasite-induced hypoglycaemia has also been attributed to increased hepatocellular damage by the parasites (Dekker *et al.*, 1997). During replication, the *Plasmodium* parasites, invade hepatic cells where the parasites undergo proliferation and morphological changes. This increased migration and replication is associated with hepatocellular damage (Dekker *et al.*, 1997). Since the liver plays a pivotal role in the breakdown of the activated insulin-receptor complex, hepatocellular damage may result in slow insulin receptor recycling, which in turn may precipitate hypoglycaemia in malaria infected rats (Onyesom & Agho, 2011). However, in the absence of histological studies, the current study cannot fully explain the mechanisms responsible for the observed reduction in blood glucose concentration by the malaria parasites.

Treatment of non-infected rats with oral CHQ twice daily, significantly decreased blood glucose concentrations. These results are in agreement with previous studies which have reported blood glucose lowering effects of CHQ (Knutson *et al.*, 1985, Davis, 1997, Jarzyna *et al.*, 2001). Some studies have attributed the CHQ-induced hypoglycaemia to increased insulin secretion (Davis, 1997) or decreased insulin degradation (Knutson *et al.*, 1985). Chloroquine is a diprotic base which rapidly accumulates in acidic lysosomes and endosomes thereby elevating lysosomal pH. This increase in lysosomal pH compromises the optimal activity of lysosomal hydrolases that are responsible for the degradation of insulin, causing an increase in plasma insulin concentration (Knutson *et al.*, 1985). Indeed, we have also demonstrated increased plasma CHQ insulin concentrations following treatment with oral CHQ (Table 2). This increase in plasma CHQ concentrations might be responsible for this reduction in blood glucose concentrations. Furthermore, some authors have linked the CHQ-induced hypoglycaemia to the inhibition of gluconeogenesis due to increased CHQ-hepatocellular damage (Jarzyna *et al.*, 2001). Jarzyna and colleagues (2001) reported the inhibition of enzymes that are involved in gluconeogenesis following oral CHQ administration. These enzymes include glutamate dehydrogenase, a key enzyme in amino

acid metabolism in the liver and kidney (Jarzyna *et al.*, 1997, Jarzyna *et al.*, 2001). The inhibition of this enzyme in turn results in a decrease in hepatic glucose output, which precipitate hypoglycaemia. The current study has demonstrated independent blood glucose lowering effects of oral CHQ

Blood glucose concentrations of *P. berghei*-infected rats treated with oral CHQ significantly decreased with a concomitant increase in plasma insulin concentrations. The current data indicates that oral CHQ-induced hypoglycaemia is in part mediated through increased plasma insulin concentrations. The data obtained in the current study indicate that both the malaria parasite and oral CHQ possess blood glucose lowering effects.

Interestingly, a once-off topical application of the pectin-CHQ patch had no significant effects on blood glucose concentrations of non-infected rats. The ability of the pectin-CHQ patch to maintain blood glucose concentrations comparable to those of the non-infected controls indicates that our novel CHQ formulation is able to avert the reported adverse effects of oral CHQ treatment on blood glucose homeostasis. Treatment of *P.berghei*-infected rats with a once-off topical application of the pectin-CHQ patch increased blood glucose concentrations by comparison with oral CHQ treated animals. This increase in blood glucose can be attributed to the continuous, increased food intake by the growing weanling rats. Interestingly, there was a significant decrease in blood glucose concentrations in animals treated with oral CHQ. When administered orally, CHQ has a bitter and unpalatable taste. As a result, the food intake by the animals is reduced which subsequently leads to a decrease in blood glucose levesl. The use of the pectin-CHQ matrix patch probably masked the bitterness of the drug; as evidenced by increased food intake (Table 2) which in turn increased blood glucose concentrations and percentage body weight of the animals treated with the pectin-CHQ matrix patch. The data presented in current study demonstrates that transdermal delivery of CHQ not only averts the adverse effects of oral CHQ on blood glucose homeostasis, but may also mask the bitter taste of CHQ. The shortfall of the study is that hepatic and muscle glycogen concentrations were not measured as this might be another mechanism that is responsible for the observed hypoglycaemia.

4.3. Effects on renal function

4.3.1. Renal effects of CHQ

Renal function studies were designed to investigate whether topically applied CHQ via the pectin-CHQ matrix patch can avert the reported oral CHQ-induced adverse effects on renal fluid and electrolyte handling. Furthermore, the current study investigated the effects of both the *Plasmodium* parasite infection and CHQ treatment on renal function in an effort to distinguish between the effects of oral and transdermal CHQ treatments on renal function. Previous reports indicated that CHQ treatment impairs kidney function, resulting in inappropriate Na^+ handling (Musabayane *et al.,* 1993) and a reduction in blood pressure (Sofola *et al.,* 1981, Musabayane *et al.,* 1999). These changes are believed to be attributed to the increased deposition of CHQ in tissues such as the heart and kidney following treatment with either oral or intravenous CHQ (McChesney *et al.,* 1976). Therefore, there is a need for a novel CHQ formulation which will minimize deposition of CHQ in these tissues. The results indicate that transdermal delivery of CHQ via the pectin-CHQ matrix patch has no effect on renal electrolyte handling and hence has the potential to maintain normal blood pressure in non-infected and *P.berghei*-infected rats. These results are suggestive of the ability of the pectin-CHQ matrix patch to provide slow, sustained CHQ releases into the circulation, thereby avoiding drug dumping in the kidneys, which might alter renal function.

Treatment of non-infected and *P.berghei*-infected rats with oral CHQ significantly increased the 24 hour urinary Na^+ output. Indeed these finding are consistent with previous studies which have reported increase sodium excretion following acute CHQ administration (Musabayane *et al.,* 1993, Musabayane *et al.,* 1996). This CHQ-induced natriuresis is thought to be mediated through increased plasma AVP concentrations (Musabayane *et al.,* 1993). CHQ increased plasma AVP without affecting the 24 hour urine output. Indeed, we have also observed increased plasma AVP concentrations following treatment with oral CHQ. CHQ supresses the vasopressin-induced increase in cyclic adenosine monophosphate (cAMP) production in isolated inner medullary collecting ducts (Musabayane *et al.,* 2000). This is suggestive of the ability of CHQ to interfere with the normal antidiuretic response to vasopressin by reducing cAMP formation (Musabayane *et al.,* 2000). These CHQ-induced

natriuretic effects are thought to be mediated through the action of AVP effects on V_1 receptors (Musabayane *et al.*, 1997), where AVP exerts pressor effects causing an increase in Na^+ excretion without changing the urine flow rate. However, in the current study we have reported a reduction in the 24 hour urine output following treatment with oral CHQ. We speculate that this phenomenon may also be attributed to the reduced water intake that was recorded in animals treated with oral CHQ. Following a once-off topical application of the pectin-CHQ matrix patch to non-infected and *P. berghei*-infected rats, the 24 hour urinary Na^+ output was comparable to that of the untreated non-infected control. These results are suggestive of the ability of the transdermal route to avert the adverse effects on renal Na^+ handling. In summary, the current data suggests that the oral CHQ natrietic effects are in part mediated through increased plasma AVP concentrations.

Urinary K^+ outputs of both untreated and oral CHQ treated *P. berghei*-infected animals were significantly increased during the treatment period. This hyperkalaemia is thought to be mediated through to increased release of K^+ from RBCs due to haemolysis. Indeed, the present study also recorded a concomitant reduction in haematocrit levels of these animals. This reduction in haematocrit values is suggestive of a decrease in the number of red blood cells due to haemolysis, therefore releasing K^+ from ruptured red blood cells. These findings are indeed in agreement with previous studies which have reported increased plasma K^+ concentrations in malaria patients (Abdalla *et al.*, 1980, Kain *et al.*, 2001). Kain and colleagues reported increased plasma K^+ in malaria patients infected with *Plasmodium vivax* treated with oral CHQ (Kain *et al.*, 2001). Following a once-off topical application of the pectin-CHQ matrix patch, the 24 hour urinary K^+ outputs of *P. berghei*-infected was increased. This increase in K^+ concentration might be attributed to RBC haemolysis by the *P. berghei* parasite rather than the pectin-CHQ matrix patch treatment. During replication, the *Plasmodium* parasite invades red blood cells and undergoes differentiation which subsequently ruptures red blood cells, resulting to the reduction of red blood cells (Phillips *et al.*, 1986, Phillips & Pasvol, 1992) and increased release of K^+ . The presence of hyperkalaemia in non-infected, patch-treated animals provides supporting evidence that transdermally delivered CHQ does not increase urinary K^+. These results suggests that the malaria parasite, rather than CHQ treatment, is responsible for the increased K^+ output in malaria patients treated with CHQ.

4.3.2. Effects of CHQ treatments on blood pressure

To distinguish between the effects of oral and transdermal CHQ treatments on MAP, non-infected and *P.berghei*-infected rats were treated with either oral CHQ or a once-off topical application of the pectin-CHQ matrix patch. In addition the current study investigated the effects of a novel CHQ formulation on MAP in an effort to avert the adverse effects on blood pressure that are reported following oral administration of CHQ.

The results of the present study demonstrate the hypotensive effects of the *P. berghei* parasites. This reduction in blood pressure is aggravated by the oral CHQ treatment. The blood pressure-lowering effects of the *Plasmodium* parasite are possibly mediated through relative bradycardia and peripheral dilation that occurs during malaria infection as a result of increased histamine production (Butler & Weber, 1973). These histamines possibly act on blood vessels where they cause vasodilation of peripheral blood vessels, resulting to a reduction in blood pressure (Farrell & David, 1988, Bethel *et al.,* 1996, Anigbogu & Olubowale, 2002). The possible mechanisms that are responsible for oral-CHQ induced reduction in blood pressure were not elucidated in the present study. However, some authors have attributed the oral CHQ hypotensive effects to the ability of the drug to decreased vascular resistance that is induced via the production of nitric oxide which is a potent vasodilator (Don-Michael & Aiwazzadeh, 1970). Indeed, we have previously reported a reduction in both blood pressure and GFR following treatment with oral CHQ (Musabayane *et al.*, 1994). However, the current study did not record any changes in GFR. This discrepancy cannot be explained by the present study. Interestingly, the once-off topically applied pectin-CHQ matrix patch had no effect on MAP. The data presented in the current study suggests that the malaria parasites and CHQ treatment work synergistically to reduce blood pressure in *P. berghein*-infected rats treated with oral CHQ. The possible mechanisms responsible for the reduction in blood pressure were not investigated in the current study

4.5. CHQ Pharmacokinetics

Chloroquine is a drug with an unusual combination of pharmacokinetic properties including a large volume of distribution (Gustafsson *et al.,* 1983) and a resulting long half-life, such that after a single dose, a plasma half-life of 3-5 days has been found (McChesney *et al.,* 1976, Sakalo & Sangodeyi, 1976). Chloroquine pharmacokinetics studies were designed to evaluate

whether transdermal CHQ formulation is able to achieve peak plasma CHQ concentrations and to sustain these CHQ plasma concentrations for a longer period. Peak plasma CHQ concentrations were achieved within a period of 24 hours following oral CHQ treatment. These findings are in agreement with previous reports which have shown rapid CHQ absorption following oral CHQ administration of this drug (Gustafsson *et al.*, 1983). A decrease in plasma CHQ concentrations observed after 24 hour of the first oral CHQ administration may be attributed to the lysosomotropic properties of this anti-malarial drug. Chloroquine has been reported to bind and accumulation in various tissues, including the heart, liver kidneys and spleen (Gustafsson *et al.*, 1983, Ursing *et al.*, 2009). The detection of CHQ in plasma 21 days after the last day of dosing is indicative of this drug's long half- life and its slow rate of elimination from the system (Gustafsson *et al.*, 1983, Gustafsson *et al.*, 1987). The plasma CHQ concentrations were reduced by 3-folds during the post treatment period, suggestive of the ability of CHQ to bind avidly to almost all the different epithelial cells and body tissues following oral administration.

The ability of the CHQ patch to attain peak CHQ concentrations comparable to that of orally administered CHQ validates the rapid delivery of drug into the systemic circulation via the transdermal route. The pectin-CHQ matrix patch formulation described in the current study sustains a prolonged release of CHQ up to 5 days. These findings are in agreement with previous reports which have shown the ability of transdermal drug delivery systems to provide sustained controlled release of drugs into the systemic circulation (Naik *et al.*, 2000, Prausnitz & Langer, 2008). Transdermally delivered CHQ was able to deliver sustained, controlled therapeutic CHQ doses which were able to clear the malaria parasites within a period of 5 days. Indeed, we have previously reported the ability of the pectin CHQ matrix patch to provided sustained CHQ release (Musabayane *et al.*, 2003). Furthermore, following treatment with oral CHQ (30 mg) twice daily, for 5 days; an average of approximately 300 mg of CHQ was administered to the animals. However, with transdermal application of the pectin-CHQ matrix patch, only 56 mg of CHQ was administered throughout the treatment period. The use of the pectin-CHQ matrix patch may alleviate the side-effects that are associated with CHQ dumping in vital tissues following oral administration of CHQ. The current study has demonstrated the feasibility of the use the pectin CHQ matrix patch for topical application of CHQ in the management of malaria.

CHAPTER 5

CONCLUSIONS

The results described in this study demonstrate that the novel transdermal CHQ-formulation is able to deliver therapeutic doses of CHQ into the systemic circulation in a sustained, controlled manner. Furthermore, the delivered doses were able to clear the *P. berghei* parasites within a period of 5 days following a once-off topical application of the pectin-CHQ patch. With the current study, we were able to distinguish between the pathophysiological effects of the *P. berghei* parasites and those of the CHQ treatments. The current data demonstrates the independent-blood glucose lowering effects of the malaria parasites and CHQ treatments. The results show that oral CHQ-elicited blood glucose lowering effects in experimental animals are in part mediated via increased plasma insulin concentrations. Interestingly, a once-off topical application of the pectin-CHQ patch was able to circumvent these adverse effects and maintained normal blood glucose concentrations. Oral administration of CHQ significantly increased the urinary Na^+ output and was associated with hyperkalaemia. The increased urinary Na^+ output was in part mediated through increased plasma AVP following oral CHQ administration. The CHQ formulation was able to circumvent these effects and did not alter the renal fluid and electrolytes handling. In addition to natriuresis and hyperkalaemia, oral CHQ administration reduced the MAP of experimental animals. These hypotensive effects of oral CHQ are thought to be mediated through CHQ-induced increased production of vasodilators such as nitric oxide. However, the present study did not elucidate the mechanisms that are responsible for the hypotensive effects of CHQ. The pectin-CHQ matrix patch did not produce any significant changes in MAP, suggestive of the ability of the CHQ formulation to deliver slow, sustained CHQ release.

In summary, the results presented in this dissertation demonstrate that the transdermal CHQ formulation does not only clear the malaria parasites from the systemic circulation, but also circumvents the adverse effects that are usually associated with oral administration of CHQ on blood glucose and renal electrolyte handling.

5.1. Shortfalls of the study

The shortfall of the current study is that liver and muscle glycogen concentrations were not measured as this might be another mechanism involved in the reduction of blood glucose concentrations that we have reported in the current study. Moreover, hepatocellular damage is

suggested as one of the mechanisms responsible for the parasite-induced hypoglycaemia. However, there were no histological studies that were done on the liver tissue to assess histological changes. This study has also established blood pressure lowering effects of both the malaria parasites and oral CHQ treatment. No studies were conducted to elucidate the possible mechanisms responsible for these hypotensive effects of oral CHQ. Another limitation of the study is that CHQ concentration in tissues such as the kidneys, liver, pancreas and heart, were not assessed. This is of importance since the oral CHQ-induced disturbances in blood glucose homeostasis and renal function are in part ascribed to increased deposition of CHQ in these tissues.

5.2. Recommendations for future studies

We have established that oral CHQ elicit adverse effects on blood glucose homeostasis and renal function. However, these effects were averted by transdermal delivery of CHQ via the pectin-CHQ patch. Future studies should, therefore, assess the deposition of CHQ in tissues such as the kidneys, liver and pancreas following oral or pectin-CHQ matrix patch treatments, as this might provide an explanation for the observed effects of oral CHQ. Future studies need also to investigate other possible mechanisms that are responsible for the reported blood glucose-lowering effects of oral CHQ. For instance studies which will measure both liver and muscle glycogen concentrations need to be conducted. We also need studies which will elucidate the possible mechanisms that are responsible for the decrease in blood pressure that was reported following oral CHQ treatment. In the present study we have reported decreased haematocrit levels in *P. berghei*-infected rats, haematocrit is a good marker for anaemia. Studies which will elucidate the possible mechanisms responsible for the reduction in haematocrit are required. For instance, measurements of hepcidin, a recently discovered peptide hormone, that plays a major role in iron regulation and is thought to play a central role in the anaemia of chronic inflammation.

CHAPTER 6
REFERENCES

1. Abdalla, S., Weatherall, D. J., Wickramasinghe, S. N. and Hughes, M. (1980) The anaemia of *P. falciparum* malaria. *British journal of Haematology* 46: 171–183.
2. Achan, J., Talisuna, A. O., Erhart, A., Yeka, A., Tibenderana, J. K., Baliraine, F. N., Rosenthal, P. J. and D'Alessandro, U. (2011) Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. *Malaria Journal* 10: 1-12.
3. Aderounmu, A. F. (1984) In vitro assessment of the antimalarial activity of chloroquine and its major metabolites. *American Journal of Tropical Medicine and Parasitology* 78: 581-585.
4. Ahmed, M. H., Balment, R. J. and Ashton, N. (2003) Renal action of acute chloroquine and paracetamol administration in the anesthetized, fluid-balanced rat. *Journal of Pharmacology and Experimental Therapeutics* 306: 478-483.
5. Amos, W. M., Sardinha, J. F., Costa, M. R., Santana, M. S., Alecrim, M. G. and Lacerda, M. V. (2010) Clinical aspects of hemolysis in patients with *P. vivax* malaria treatedwith primaquine, in the Brazilian Amazon. *Brazil Journal of Infectious Diseases* 14: 410-412.
6. Angus, B. J., Chotivanich, K., Udomsangpetch, R. and White, N. J. (1997) In vivo removal of malaria parasites from red blood cells without their destruction in acute *falciparum* malaria. *Blood* 90: 2037–2040.
7. Anigbogu, C. N. and Olubowale, O. A. (2002) Effects of Malaria on Blood Pressure, Heart Rate, Electrocardiogram and Cardiovascular Response to Change in Posture. *Nigerian Quarterly Journal of Hospital Medicine* 12: 17-20.
8. Bagot, S., Boubou, M. I., Campino, S., Behrschmidt, C., Gorgette, O., Guénet, J.-L., Penha-Gonçalves, C., Mazier, D., Pied, S. and Cazenave, P. A. (2002) Susceptibility to Experimental Cerebral Malaria Induced by *Plasmodium berghei* ANKA in Inbred Mouse Strains Recently Derived from Wild Stock. *Infection and Immunity* 70: 2049–2056.
9. Baguet, J. P. and Fabre, F. T. (1999) Chloroquine cardiomyopathy with conduction disorders. *Heart* 81: 221-223.
10. Baird, J. K. and Hoffman, S. L. (2004) Primaquine Therapy for Malaria. *Clinical Infectious Diseases* 39: 1336-1345.

11. Barry, B. W. (2001) Novel mechanisms and devices to enable successful transdermal drug delivery. *European Journal of Pharmaceutical sciences* 14: 101-114.
12. Bethel, D. B., Phuong, P. T., Phuon, N. C. X. T., Nosten, F., Waller, D., Davis, T. M. E., Day, N. P. J., Crawley, J., Brewste, D., Pukrittayakamee, S. and White, N. J. (1996) Electrocardiographic monitoring in severe *falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 90: 266-269.
13. Bjo¨rkman, A. (2002) Malaria associated anaemia, drug resistance and antimalarial combination therapy. *International Journal for Parasitology* 32: 1637–1643.
14. Butler, T. and Weber, D. M. (1973) On the nature of orthostatic hypotension in acute malaria. *The American Journal of Tropical Medicine and Hygiene* 22: 439-442.
15. Chi, S. C., Park, E. S. and Kim, H. (1995) Effect of penetration enhancers on flurbiprofen permeation through rat skin. *International Journal of Pharmaceutics* 126: 267–274.
16. Chien, Y. W. (1992) Novel drug delivery systems. *Drugs and the Pharmaceutical Sciences* 50: 797.
17. Clark, I. A. and Cowden, W. B. (2003) The pathophysiology of falciparum malaria. *Pharmacology & therapeutics* 99: 221-260.
18. Clark, I. A. and Schofield, L. (2000) Pathogenesis of Malaria. *Parasitology Today* 16: 451-454.
19. Cohuet, A., Osta, M. A., Morlais, I., Awono-Ambene, P. H., Michel, K., Simard, F., Christophides, G. K., Fontenille, D. and Kafatos, F. C. (2006) Anopheles and Plasmodium: from laboratory models to natural systems in the field. *European Molecular Biology Organization Reports* 7: 1285 - 1289.
20. Das, B. S. (2008.) Renal failure in malaria. *Journal of Vector Borne Diseases* 45: 83-97.
21. Davis, T. M. (1997) Antimalarial drugs and glucose metabolism. *British Journal of Clinical Pharmacology* 44: 1-7.
22. Dekker, E., Hellersstein, M. K., Romijn, J. A., Neese, R. A., Peshu, N., Endert, E., Marsh, K. and 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.
23. Don-Michael, T. and Aiwazzadeh, S. (1970) The effects of acute chloroquine poisoning with special reference to the heart. *American Heart Journal* 79: 831-842.

24. Dondo, F. and Mubagwa, K. (1990) Chloroquine interacts with muscarinic receptors and inhibits cholinergic effects in the heart. *African Journal of Medical science* 19: 237-243.
25. Drakesmith, H. and Prentice, A. M. (2012) Hepcidin and the Iron-Infection Axis. *Science* 338: 768-772.
26. Durcharme and Farinotti. (1996).
27. Elias, P. M. J. (1983) Journal of Investigative Dermatology. *Epidermal lipids, barrier function and desquamation* 80: 44–49.
28. Elsheikha, H. M. and Sheashaa, H. A. (2007) Epidemiology, pathophysiology, management and outcome of renal dysfunction associated with plasmodia infection. *Parasitology Research* 101 1183-1190.
29. Etim, O. E., Ekaidem, I. S., Akpan, E. J., Usoh, I. F. and Akpan, H. D. (2011) Changes in electrolyte levels in uncoplicated *Plasmodium falciparum* malaria: The effects on quinine therapy. *Continental J. Pharmacology and Toxicology Research* 4.
30. Farrell, M. P. and David, A. S. (1988) Severe orthostatic hypotension during treatment of *falciparum* malaria. *British Medical Journal* 296: 396.
31. Fernando, D., Rodrigo, C. and Rajapakse, S. (2011) Primaquine in *vivax* malaria: an update and review on management issues. *Malaria Journal* 10: 1-12.
32. Fernando, S. D., Rodrigo, C. and Rajapakse, S. (2011) Chemoprophylaxis in malaria: drugs, evidence of efficacy and costs. *Asian Pacific Journal of Tropical Medicine* 4: 330-336.
33. Finnin, B. C. and Morgan, T. M. (1999) Transdermal Penetration Enhancers: Applications, Limitations, and Potential. *Journal of Pharmaceutical Sciences* 88: 955-958.
34. Gondwe, M. L., Kamadyaapa, D. R., Tufts, M. A., Chuturgoon, A. A., Ojewole, J. A. O. and Musabayane, C. T. (2008) Effects of Persea americana (Mill) [Lauraceae]["Avocado"] ethanolic extract on blood glucose and kidney function in streptozotocin-induced diabetic rats and on kidney cell lines of the proximal (LLC-PK1) and distal tubules (MDBK). *Methods and Findings in Experimental and Clinical Pharmacology and Therapeutics* 30: 25-35.
35. Goodman, A. L., Forbes, E. K., Williams, A. R., Douglas, A. D., de Cassan, S. C., Bauza, K., Biswas, S., Dicks, M. D. J., Llewellyn, D., Moore, A. C., Janse, C. J., Franke-Fayard, B. M., Gilbert, S. C., Hill, A. V. S., Pleass, R. J. and Draper, S. J.

(2013) The utility of *Plasmodium berghei* as a rodent model for anti-merozoite malaria vaccine assessment. *Scientific Reports* 3: 1-13.

36. Grant, G. T. (1973) Biological interactions between polysaccharides and divalent cations. *FEBS Letters* 32: 195-198.
37. Greenwood, B. and Mutabingwa, T. (2002) Malaria in 2002. *Nature* 415: 670-672.
38. Gumede, B., Folbb, P. and Ryffela, B. (2003) Oral artesunate prevents Plasmodium berghei Anka infection in mice. *Parasitology International* 52: 53–59.
39. Gustafsson, L. L., Lindstrom, B., Grahnen, A. and Alvan, G. (1987) Chloroquine excretion following malaria prophylaxis. *British Journal of Clinical Pharmacology* 24: 221-224.
40. Gustafsson, L. L., Walker, O., Alvan, G., Beermann, B., Estevez, F., Gleisner, L., Lindstrom, B. and Sjoqvist, F. (1983) Disposition of chloroquine in man after single intravenous and oral doses. *British Journal of Clinical Pharmacology* 15: 471-479.
41. Hemingway, J., Field, L. and Vontas, J. (2002) An overview of insecticide resistance. *Science* 298: 96-97.
42. Howard, C. T., McKakpo , U. S., Quakyi, I. A., Bosompem, K. M., Addison, E. A., Sun, K., Sullivan, D. and Semba, R. D. (2007) Relationship of hepcidin with parasitemia and anaemia among patients with uncomplicated *Plasmodium falciparum* Malaria in Ghana. *Blood* 121: 3016-3022.
43. Jackson, S. M., Williams, M. L., Feingold, K. R. and Elias, P. M. (1993) Pathobiology of the stratum corneum. *Western Journal of Medicine* 158: 279-285.
44. Janse, C. J. and Waters, A. P. (1995) *Plasmodium berghei*: The Application of Cultivation and Purification Techniques to Molecular Studies of Malaria Parasites. *Parasitology Today* 11: 138-143.
45. Jarzyna, P., Kiersztan A, L. O. and J., B. (2001) The inhibition of gluconeogenesis by chloroquine contributes to its hypoglycaemic action. *European Journal of Pharmacology* 428: 381-388.
46. Jarzyna, R., Lenarcik, E. and Bryła, J. C. (1997) Chloroquine is a potent inhibitor of glutamate dehydrogenase in liver and kidney-cortex of rabbit. *Pharmacological Research* 35: 79-84.
47. Kain, K. C., MacPherson, D. W., Kelton, T., Keystone, J. S., Mendelson, J. and MacLean, J. D. (2001) Malaria deaths in visitors to Canada and in Canadian travellers: a case series. *Canadian Medical Association* 164: 654-659.

48. Kanitakis, J. (2002) Anatomy, histology and immunohistochemistry of normal human skin. *European Journal of Dermatology* 12: 390-401.
49. Knutson, V. P., Ronnet, G. V. and 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: 114180-114188.
50. Kolarsick, P. A. J., Maria Ann Kolarsick, B. S. and Goodwin, C. (2011) Anatomy and Physiology of the Skin. *Dermatology Nurses' Association* 3: 203-213.
51. Krishna, S., Taylor, A. M., Supanaranond, W., Pukrittayakemee, S., Ter Kuile, F., Tawfiq, K. M., Holloway, P. A. and White, N. J. (1999) Thiamine deficiency and malaria in adults from southeast Asia. *Lancet* 353: 546-549.
52. le Sueur, D., Sharp, B. L., Gouw, E. and Ngxongo, S. (1996) Malaria in South Africa. *South African Medical Journal* 86: 936-939.
53. Lenz and Holzer. (1984).
54. Looareesuwan, S., J.White, N. and Chanthavanich, P. (1986) Cardiovascular toxicity and distribution kinetics of intravenous chloroquine. *Br J Clin Pharmacol* 22: 31–36.
55. Looareesuwan, S., Vanijanonta, S., Viravan, C., Wilairatana, P., Charoenlarp, P. and Andrial, M. A. (1994) Randomized trial of mefloquine alone and artesunate followed by mefloquine for the treatment of acute uncomplicated *falciparum* malaria. *Tropical Medicine and Parasitology* 88: 131–136.
56. Mackintosh, C. L., J.G.Beeson and Marsh, K. (2004) Clinical features and pathogenesis of severe malaria. *TRENDS in Parasitology* 20.
57. May, C. D. (1990) Industrial pectins: Sources, production and applications. *Carbohydrate Polymers* 12: 79-99.
58. McChesney, E. W., Banks, W. F. J. and Fabian, R. J. (1976) Tissue distribution of chloroquine, hydroxychloroquine, and desethylchloroquine in the rat. *Toxicology and Applied Pharmacology* 10: 501-513.
59. McDevitt, M. A., Xie, J., Gordeuk, V. and Bucala R. (2004) The anemia of malaria infection: role of inflammatory cytokines. *Current Hematology* 3: 97–106.
60. Mehta, R. (2004) Topical and Transdermal Drug Delivery: What a Pharmacist Needs to Know. *The Accreditation Council for Pharmacy Education (ACPE)*.
61. Mellman. (1986).
62. Meshnick, S. R. (2002) Artemisinin: mechanisms of action, resistance and toxicity. *International Journal for Parasitology* 32: 1655-1660.

63. Meshnick, S. R., Taylor, T. E. and Kamchonwongpaisan, S. (1996) Artemisinin and the Antimalarial Endoperoxides: from Herbal Remedy to Targeted Chemotherapy: Microbiological Review. *Microbiological Reviews*: 301-315.

64. Munjeri, O., Hodza, P., Osim, E. E. and 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: 905-908.

65. Murphy, G. F. (1997) Histology of the skin. *Lever's Histology of the Skin*: 5-45.

66. Musabayane, C. T., Cooper, R. G., Rao, P. V. V. and Blment, R. J. (2000b) Effects of ethanol on the changes in renal fluid and electrolyte handling and kidney morphology induced by long-term chloroquine administration. *Elsevier* 22: 129-138.

67. Musabayane, C. T., Forsling, M. L. and Balment, R. J. (1997) Arginine vasopressin increases renal sodium excretion in the anesthetized rat through V1 receptors. *Renal Failure* 19: 23-32.

68. Musabayane, C. T., Gondwe, M., Kamadyaapa, D. R., Chuturgoon, A. A. and 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: 389-397.

69. Musabayane, C. T., Munjeri, O. and Matavire, T. P. (2003) Transdermal Delivery of Chloroquine by Amidated Pectin Hydrogel Matrix Patch in the Rat. *Renal Failure* 25: 525-534.

70. Musabayane, C. T., Munjeri, O. and Matavire, T. P. (2003) Transdermal delivery of chloroquine by amidated pectin hydrogel matrix patch in the rats. *Renal failure* 25: 525-534.

71. Musabayane, C. T., Musvibe, A., Wenyika, J., Munjeri, O. and Osim, E. E. (1999) Chloroquine influences renal function in rural Zimbabweans with acute transient fever. *Renal Failure* 21: 189-197.

72. Musabayane, C. T., Ndhlovu, C. E. and Balment, R. J. (1994) The effects of oral chloroquine administration on kidney function. *Renal Failure* 16: 221-228.

73. Musabayane, C. T., Ndhlovu, C. E., Mamutse, G., Bwititi, P. and Balment, R. J. (1993) Acute chloroquine administration increases renal sodium excretion *Journal of Tropical Medicine and Hygiene* 96: 305-310.

74. Musabayane, C. T., Wargent, E. T. and Balment, R. J. (2000) Chloroquine inhibits arginine vasopressin production in isolated rat inner medullary segments induced cAMP collecting duct. *Renal Failure* 22: 27-37.

75. Musabayane, C. T., Windle, R. J., Forsling, M. L. and Balmet, R. J. (1996) Arginine vasopressin mediates the chloroquine induced increase in renal sodium excretion. *Tropical Medicine and International Health* 1: 542-550.

76. Naik, A., Kalia, Y. N. and Guy, R. H. (2000) Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical Science & Technology Today* 3: 318-326.

77. Oakenfull, D. G. (1991) The chemistry of high-methoxyl pectins. *In The chemistry and technology of pectin, ed. R.H. Walter. (New York: Academic Press)*

78. Onyesom, I. and 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.

79. Owens, R. C., Jr. and Nolin, T. D. (2006) Antimicrobial-associated QT interval prolongation: pointes of interest. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 43: 1603-1611.

80. Pathan, I. B. and Setty, C. M. (2009) Chemical Penetration Enhancers for Transdermal Drug Delivery Systems. *Tropical Journal of Pharmaceutical Research* 8: 173-179.

81. Phillips, R. E., Looareesuwan, S., Warrell, D. A., Lee, S. H., Karbwang, J., White, N. J., Swasdichai, C. and Weatherall, D. J. (1986) The importance of anaemia in cerebral and uncomplicated *falciparum malaria*: role of complications, dyserythropoiesis and iron sequestration. *Journal of Medicine* 58: 305–323.

82. Phillips, R. E. and Pasvol, G. (1992) Anaemia of *Plasmodium falciparum* malaria *Baillière's Clinical. Haematology* 5: 315–330.

83. Poole and Ohkuma. (1981).

84. Prausnitz, M. R. and Langer, R. (2008) Transdermal drug delivery. *Nature Biotechnology* 26: 1261-1268.

85. Pussard, E. and Verdier, F. (1994) Antimalarial 4-aminoquinolines: mode of action and pharmacokinetics. *Fundamental & Clinical Pharmacology* 8: 1-17.

86. Sakalo, L. A. and Sangodeyi, J. M. (1976) Effect of chloroquine on isolated quine-pig atria *West African Journal of Pharmacology Research* 3: : 85-86.

87. Sanguinetti, M. C. and Jurkiewicz, N. K. (1990) Two components of cardiac delayed rectifier K+ current. Differential sensitivity to block by class III antiarrhythmic agents. *The Journal of general physiology* 96: 195-215.

88. Shah, R. R. (2005) Drugs, QTc interval prolongation and final ICH E14 guideline : an important milestone with challenges ahead. *Drug safety : an international journal of medical toxicology and drug experience* 28: 1009-1028.

89. Sinha, V. R. and Kaur, P. M. (2000) Permeation Enhancers for Transdermal Drug Delivery. *Drug Development and Industrial Pharmacy* 26: 1131–1140.

90. Sintov, A., Ze'evi, A., Uzan, R. and 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 : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* 47: 299-303.

91. Slutsker, L., Taylor, T. E., Wirima, J. J. and Steketee, R. W. (1994) In-hospital morbidity and mortality due to malaria-associated severe anaemia in two areas of Malawi with different patterns of malaria infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 88: 548–551.

92. Sofola, O. A., Olude, I. O. and Adegoke, F. (1981) The effects of chronic chloroquine toxicity on blood pressure in rats. *Journal of Tropical Medicine and Hygiene* 84: 249-252.

93. Sriamornsak, P. (2003) Chemistry of Pectin and Its Pharmaceutical Uses : A Review. *Silpakorn University International Journal* 3: 206-228.

94. Tanojo, H., BosvanGeest, A., Bouwstra, J. A., Junginger, H. E. and Bodde, H. E. (1997) In vitro human skin barrier perturbation by oleic acid: thermal analysis and freeze fracture electron microscopy studies. *Thermochim. Acta* 293: 77-85.

95. Taylor, T. E., Molyneux, M. E., Wirima, J. J., Fletcher, K. A. and Morris, K. (1988) Blood glucose levels in Malawian children before and during the administration of intravenous quinine for severe *falciparum* malaria. *New England Journal of Medicine* 319: 1040–1047.

96. Thiele, J. J. and Ekanayake-Mudiyanselage, S. (2007) Vitamin E in human skin: organ-specific physiology and considerations for its use in dermatology. *Molecular aspects of medicine* 28: 646-667.

97. Tuteja, R. (2007) Malaria- An overview *Federation of European Biochemical Societies Journal* 274: 4670-4679.

98. Ursing, J., Kofoed, P., Rodrigues, A., Berqcist, Y. and Rombo, L. (2009) Chloroquine Is Grossly Overdosed and Overused but Well Tolerated in Guinea-Bissau. *Antimicrobial Agents and Chemotherapy* 53: 180-185.

99. Walker, O., Dawodu, A. H., Adeyokunn, A. A., Salako, L. A. and Alvan, G. (1983) Plasma chloroquine and desethylchloroquine concentrions in children and after chloroquine treatment for malaria *British journal of clinical pharmacology* 16: 701-705.

100. Wellems, T. E. and Plowe, C. V. (2001) Chloroquine resistant Malaria. *The Journal of Infectious Diseases* 184 770-776.

101. Wells, T. N. C., Alonso, P. L. and Gutteridge, W. E. (2009) New medicines to improve control and contribute to the eradication of malaria. *Nature Review Drug discovery* 8 879-891.

102. White, N. J. (2004) Antimalarial drug resistance. *The Journal of Clinical Investigation* 113: 1084 - 1092.

103. White, N. J., Miller, K. D., Marsh, K., Turner, R. C., Berry, C. D., Williamson, D. H. and Brown, J. (1987) Hypoglycaemia in African children with severe malaria. *Lancet* 1: 707-711.

104. White, N. J., Warrell, D. A., Chanthavanich, P., Looareesuwan, S., Warrell, M. J., Krishna, S., Williamson, D. H. and Turner, R. C. (1983) Severe hypoglycemia and hyperinsulinemia *falciparum* malaria. *New England Journal of Medicine* 309: 61-66.

105. WHO. (2003) Assessment of the safety of artemisinin compounds in pregnancy, report of two informal consultations by WHO in 2002.

106. WHO. (2000) Malaria a global crisis. *Geneva: World Health Organization*.

107. WHO. (2013) Malaria guidlines; media centre.

108. WHO. (2000) Severe and complicated malaria. *Transactions of Royal Society of Tropical Medicine and Hygiene* 94: 1-90.

109. Williams, A. C. and Barry, B. W. (2012) Penetration enhancers. *Advanced Drug Delivery Reviews* 64: 128–137.

110. Wilson, F. and Dietschy, J. (1974) The intestinal unstirred water layer: its surface area and effect on active transport kinetics. *Biochimica et Biophysica Acta* 34: 1034.

111. Zhang, J., Krugliak, M. and Ginsburg, H. (1999) The fate of ferriprotorphyrin IX in malaria infected erythrocytes in conjunction with the mode of action of antimalarial drugs. *Molecular and Biochemical Parasitology* 99: 129–141.

Appendix I

UNIVERSITY OF
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INYUVESI
YAKWAZULU-NATALI

Research Office
Animal Ethics Research Committee

Govan Mbeki Centre, Westville Campus,
University Road, Chiltern Hills, Westville, 3629, South Africa
Telephone 27 (031) 260-2273/35 Fax (031) 260-2384
Email: animalethics@ukzn.ac.za

7 June 2012

Reference: 088/12/Animal

Miss H Sibiya
School of Laboratory Medicine and
Medical Sciences
University of KwaZulu-Natal
Westville Campus

Dear Miss Sibiya

Ethical Approval of Research Project on Animals

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 2012 on the following project:

"Studies on selected chloroquine patch characteristics: stability, tissue deposition and pharmacokinetic profiles."

Yours sincerely

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

Cc Registrar, Prof. J Meyerowitz
Research Office, Dr S Soobramoney
Supervisor, Prof. C Musabayane
Dean & HOS, Prof. W Daniels
BRU, Dr S Singh

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- Howard College
- Medical School
- Pietermaritzburg
- Westville

Appendix II

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Research Office
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University Road, Chiltern Hills, Westville, 3629, South Africa
Telephone 27 (031) 260-2273/35 Fax (031) 260-2384
Email: animalethics@ukzn.ac.za

20 December 2012

Reference: 057/13/Animal

Ms H Sibiya
School of Laboratory Medicine
and Medical Sciences
University of KwaZulu-Natal
WESTVILLE Campus

Dear Ms Sibiya

RENEWAL: Ethical Approval of Research Projects on Animals

I have pleasure in informing you that the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for **2013** on the following project:

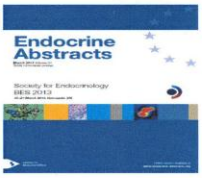
"Studies on selected chloroquine patch characteristics: stability, tissue deposition and pharmacokinetics."

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc Registrar – Prof. J Meyerowitz
Research Office – Dr N Singh
Supervisor – Prof. C Musabayane
Head of School – Prof. W Daniels
BRU, Dr S Singh

Appendix III (a)



Evaluation of efficacy of transdermal delivery of chloroquine on *Plasmodium berghei*-infected male Sprague–Dawley rats and effects on blood glucose and renal electrolyte handling

[1]Cephas T Musabayane ,[2]Samson Mukaratirwa, [1]Happiness Sibiya and [1]Pretty Murambiwa

School of [1]Laboratory Medicine and Medical Science, & [2]Life Sciences, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa

Chloroquine (CHQ) the most frequently used drug for *falciparum* malaria in sub-Saharan Africa countries evokes adverse effects on glucose homeostasis and kidney function in African children. The complications can partly be ascribed to transiently high plasma CHQ concentrations following oral administration and/or malaria parasites. We have, however, reported that topical application of the pectin CHQ matrix patch releases CHQ into the bloodstream. Accordingly, the current study determined whether CHQ delivered via the transdermal route can reduce malaria parasites and ameliorate the side effects associated with oral CHQ. The method of CHQ patch production is similar to that previously reported. Oral glucose tolerance responses (OGT) to CHQ delivered orally or transdermally were monitored in groups of non-infected and *Plasmodium berghei*-infected male Sprague–Dawley rats following glucose load. Blood glucose concentrations were measured at 15-min intervals for the first hour and hourly thereafter for 3 h. Parasitaemia, plasma insulin, blood glucose and renal function were monitored over a 21-day period divided into pre-treatment (days 0–7), treatment (days 8–12) and post-treatment (days 13–21) in separate groups following a once off application of the CHQ patch (53 mg/kg) twice daily administration of CHQ (60 mg/kg, p.o.) during the treatment period. Transdermally delivered CHQ sustained plasma concentrations of CHQ and equally reduced *P. berghei* parasites by comparison with twice daily oral chloroquine. Compared with respective control groups, OGT responses of animals administered oral and transdermal CHQ were lower at all the time points that blood was sampled after the glucose load. Oral CHQ administration increased plasma insulin concentration whilst topical CHQ patch did not have any significant effect. Oral CHQ treatment was associated with increased urinary Na^+ outputs and hyperkalaemia. The CHQ matrix patch did not influence these parameters. We conclude that the CHQ patch has the potential circumvent the adverse effects of oral CHQ.

Declaration of funding: This study was partly funded by the NRF South Africa and the University of KwaZulu-Natal, Research Division.

Appendix III (b)

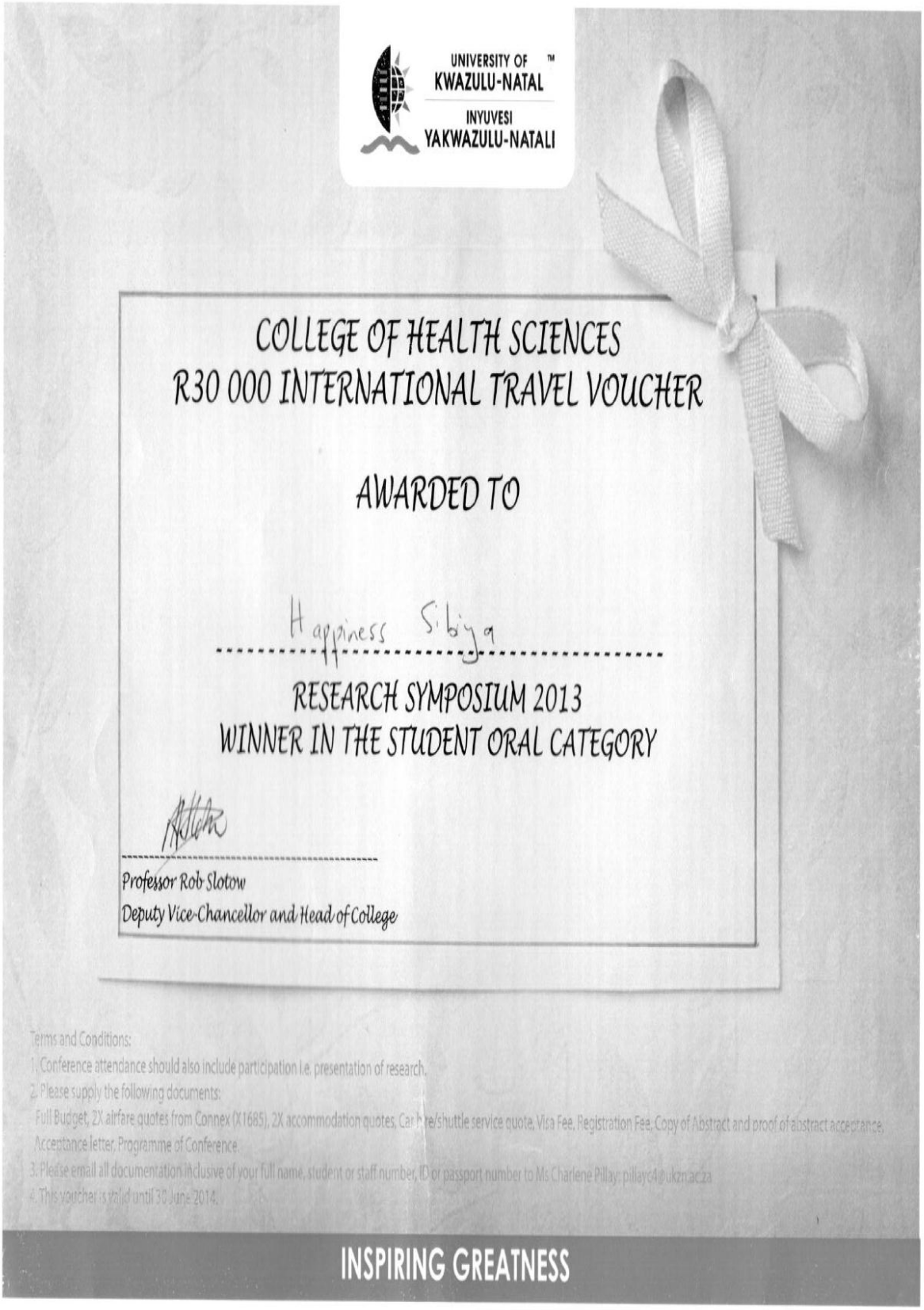
COLLEGE OF HEALTH SCIENCES RESEARCH SYMPOSIUM 2013
12-13 SEPTEMBER
K-RITH TOWER BUILDING

EVALUATION OF THE EFFICACY OF TRANSDERMAL DELIVERY OF CHLOROQUINE ON *PLASMODIUM BERGHEI*-INFECTED MALE SPRAGUE-DAWLEY RATS: EFFECTS ON BLOOD GLUCOSE AND RENAL ELECTROLYTE HANDLING

[*]**Sibiya, H,**[*] Mbatha, B., [#]Mukaratirwa, S., [*]Musabayane, C.T.

School of *Laboratory Medicine and Medical Science, & [#]Life Sciences, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa

Oral administration of chloroquine (CHQ) evokes adverse physiological effects on glucose homeostasis and kidney function in African children due to transiently high plasma CHQ concentration or malaria parasites. We have observed that oral administration of amidated pectin CHQ beads formulation sustains controlled release of CHQ into the blood stream and speculated that topical application of pectin-CHQ patch sustains controlled CHQ release into the bloodstream to perhaps ameliorate these adverse effects. Therefore, we investigated whether CHQ delivered via the transdermal route can reduce malaria parasites and avert the pathophysiological effects associated with oral CHQ administration. The pectin-CHQ patch was prepared by dissolving 5g of CHQ and 4.4g of pectin in distilled water. Parasitaemia, blood glucose and renal function were monitored in groups of control and Plasmodium berghei-infected male Sprague-Dawley rats following oral (30mg/kg, p.o) or transdermal delivery of CHQ (28mg/kg) over a 21-day period divided into pre-treatment, treatment (days 8-12) and post-treatment (days 13-21). Transdermally delivered CHQ successfully reduced P. berghei parasites from 38.72±1.25 2% to undetected levels within 5 days of treatment. Oral CHQ treatment significantly decreased blood glucose concentrations from 5.73±0.36 mmol/l to 2.93 mmol/l whilst the pectin-CHQ did not alter blood glucose levels (5.98±0.13). Oral CHQ treatment increased urinary Na^+ outputs from 170.60±7.20 to 241.9±16.38 mmol/l in comparison to the control (158.4±10.74 to 155.20±9.03) while the pectin-CHQ patch had no significant effect. We conclude that the CHQ patch has the potential avert the adverse effects on glucose homeostasis and renal function associated with oral administration of CHQ.



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