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

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INTRODUCTION

African children with severe *Plasmodium falciparum* malaria often present with metabolic complications which include impairment of glucose homeostasis, cardiovascular and kidney functions partly ascribed to *P. falciparum* infection and/or drugs used manage malaria [1].

The traditional oral chloroquine (CHQ) dosin¬g schedule of 4 tablets, equivalent to 600 mg active base, on day 1, followed by 2 tablets, equivalent to 300 mg active base, 6 hours later, and 2 tablets, 300 mg, a day for the next 2 days, giving a total of 1200 mg contributes to high plasma CHQ concentrations.

Alternative methods of CHQ delivery have, therefore, been suggested, in an effort to modify the dosing schedule which may improve patient comfort and compliance.

Against this background, literature evidence indicates that transdermal drug delivery provides sustained controlled release of drugs directly into the systemic circulation [2].

OBJECTIVES

- To develop a novel CHQ-formulation that delivers sustained slow CHQ into the systemic circulation.
- To investigate the ability of this CHQ formulation to clear the malaria parasites in *Plasmodium berghei*-infected male Sprague-Dawley rats.
- Evaluate the effects of the transdermal delivery of CHQ on renal function and on blood glucose homeostasis in malaria infected rats.

MATERIALS AND METHODS

Patch preparation
Pectin was dissolved in deionized water followed by adding CHQ, DMSO and antioxidants. After freezing, $CaCl_2$ was added for cross-linking and patch formation. The patches were then stored in the refrigerator at 2°C until use.

Determination of the amount of CHQ in patches
The amount of CHQ in the CHQ pectin patch was determined spectrophotometrically after dissolving the patch of known areas in de-ionized water. The blank contained CHQ free pectin patches in de-ionized water.

Malaria induction
Malaria was induced in male Sprague-Dawley rats (90-190 g body weight) with a single intraperitoneal injection of *P. berghei* (105 parasitised red blood cells).

Experimental design
Male Sprague-Dawley rats were divided into non-infected (control) and *P. berghei* infected groups (n=6 in each group). The groups were further subdivided into those treated orally with CHQ diphosphate powder or transdermally applied Pectin CHQ patch formulations for assessment of the effects on parasitaemia, blood glucose and renal functions.

Sub-chronic effects of CHQ
Oral CHQ was administered twice daily (60 mg/kg, p.o.) by means of a bulbed steel needle whilst the CHQ patch (53 mg/kg) was applied once at the beginning of treatment period.
The 21 day studies were divided into pre-treatment (days 0-7), treatment (days 8-12) and post treatment (days 13-21) periods.

Parasitaemia was monitored by microscopic counting of Giemsa-stained thin blood smears. A drop of blood from the tail was used to prepare blood smears.

Blood glucose and renal function were monitored throughout the 21-day experimental period.

Plasma insulin concentrations were measured from blood samples collected on day 7 of the pre-treatment period and day 8, 12 of the treatment period.

Terminal studies
To assess the effects of oral and transdermal CHQ treatments on some biochemical parameters, plasma CHQ profiles, electrolytes, AVP and insulin concentrations, separate groups of non-infected and *P. berghei* infected animals (n=6) were sacrificed on the last day treatment and post-treatment period.

Statistical analysis
All data presented as means ±SEM. Graph Pad Instat software (version 5) using one way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test was used. P values < 0.05 were considered significant.

RESULTS

Chloroquine loading efficiency
The amidated CHQ pectin patches contained 15.9 mg/ml translating into a dose of 53 mg/kg for a 300 g rat. The percentage CHQ incorporation into the matrix patch was 74%.

Effects of CHQ treatments
Parasitaemia
Figure 1 shows that the peak percentage parasitaemia of control animals was reached at day 14. The control animals were sacrificed on day 14 of the experimental period based on preliminary results. As such, all the subsequent results showing the infected control animals will be having no post treatment period (days 13-21).

Topical application of the pectin CHQ hydrogel matrix patch on the skin as well as oral CHQ equally reduced *P. berghei* parasites to levels that were undetectable by day 5 of treatment (Table 1 and Figure 1).

Table 1: Comparison of *P. berghei* parasites profiles in animals treated with oral CHQ or transdermal CHQ (n=6 in each group).

Group	Control	Oral CHQ	Transdermal CHQ
Day post treatment	*% parasitaemia*	*% parasitaemia*	*% parasitaemia*
0	26 ±2	25 ±2	26±2
3	40±3	9±2	0
5	50±17	0	0

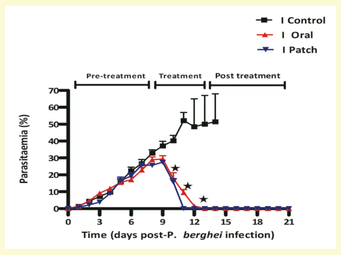

Figure 1: Comparison of percentage parasitaemia in animals treated twice daily with oral CHQ (60 mg/kg) and those treated with a single topically applied CHQ patch (53 mg/kg) with control animals. ★p<0.05 by comparison with control animals.

Blood glucose
The blood glucose concentrations of untreated non-infected animals (range 5.95 ± 0.17 to 6.80 ± 0.24 mmol/L) were significantly elevated when compared to levels in untreated *P. berghei*-infected rats (Figure 2).

Oral CHQ treatment significantly decreased blood glucose concentrations of both the non-infected and infected animals. Interestingly, the once-off topical application of the pectin-CHQ patch increased blood glucose concentrations of *P. berghei*-infected animals (Figure 2B).

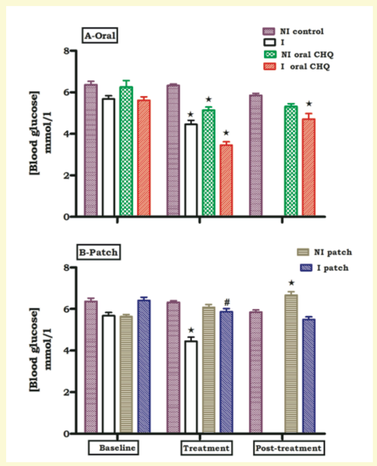

Figure 2: The effects of oral CHQ (60 mg/kg) treatment (A) and single topical application of CHQ matrix patch (53 mg/kg) (B) on blood glucose concentration. ★p<0.05 by comparison to baseline control animals. # p<0.05 by comparison control animals

Plasma insulin concentrations
Both oral and transdermal CHQ administration increased the plasma insulin concentrations of non-infected and *P. berghei* rats by comparison with respective baseline values (Figure 3).

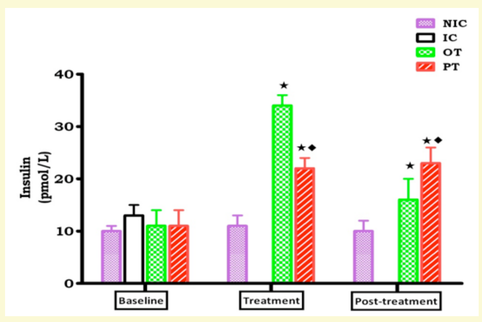

Figure 3: Comparison of the effects of oral CHQ treatment (OT) and single topical application of CHQ matrix patch (PT) on plasma insulin concentrations with respective control animals.
★ p<0.05 by comparison to respective baseline values.
◆ p<0.05 by comparison with oral CHQ treated animals.

Renal function
Treatment of non-infected animals and infected animals with oral CHQ significantly increased the 24 hour urinary Na^+ outputs (Figure 4).

CHQ matrix patch did not alter urinary Na^+ outputs of both groups of animals.

Treatment of both non infected and *P. berghei* infected animals with oral CHQ significantly increased the urinary K^+ outputs by comparison to the non-infected control animals.

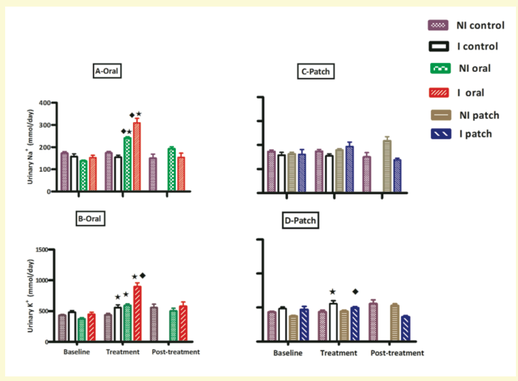

Figure 4: Comparison of the effects of oral CHQ treatment (OT) and single topical application of CHQ matrix patch (PT) on renal Na^+ and K^+ handling.
★=p<0.05 by comparison with respective baseline values.
◆= p<0.05 by comparison with oral CHQ treated animals.

CHQ pharmacokinetics
The plasma CHQ concentration measured in blood samples collected at 0.25, 0.5, 1, 2, 3 & 5 days after treatment in rats treated orally administered twice daily with CHQ (60 mg/ kg) or applied pectin CHQ hydrogel patch (53 mg/kg) on the skin for 1 day are shown in Figure 5.
Between groups comparisons indicated that the plasma CHQ concentration vs. time profiles in treated animals could not be statistically differentiated from 0.5 to 5 days and 0.5 to 4 days following oral CHQ or CHQ patch, respectively.

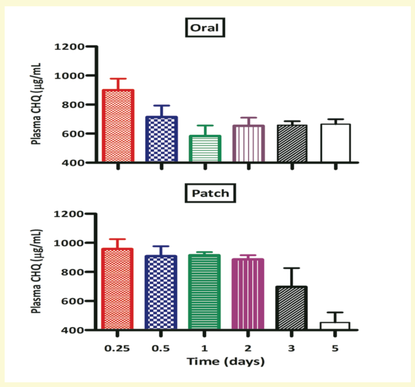

Figure 5: Plasma CHQ concentration profiles following oral administration of oral transdermal CHQ administration. Each point represents the mean ± SEM of six animals.

Plasma AVP and biochemical parameters
The plasma K^+ concentrations of animals treated twice daily with oral CHQ were significantly elevated when compared with pectin CHQ matrix patch treated animals (Table 2).
Oral CHQ treatment significantly ($p < 0.05$) increased plasma AVP by comparison to topical applied pectin-CHQ matrix patch.

Table 2: The effects of CHQ treatments on plasma AVP and electrolyte concentrations (n=6 in each group)

		Group	
Measurements	**Period**	**Oral**	**Patch**
AVP ($pmol.L^{-1}$)	Treatment	584 ± 29	496 ± 48*
Na^+ ($mmol.L^{-1}$)	Treatment	140 ± 4	141 ± 2
K^+ ($mmol.L^{-1}$)	Pre-treatment	5.09 ± 0.33	5.49 ± 0.40
	Treatment	9.96 ±1.86*#	5.38 ± 0.41* #
GFR ($ml.min\ 100g^{-1}$)	Treatment	0.90 ± 0.01	0.89 ± 0.03
Kidney mass ($g.100g^{-1}$ b.wt)	Treatment	0.93 ± 0.04	0.93 ± 0.05

* =p<0.05 by comparison to oral CHQ treated rats.
*=p< 0.05 by comparison with oral treatment values
#=p<0.05 by comparison with pre-treatment values

DISCUSSION AND CONCLUSIONS

The current study has demonstrated the sustained controlled release of CHQ from the pectin matrix patch, demonstrating the therapeutic ability to clear *P. berghei* malaria parasites from systemic circulation. The once off application of the CHQ patch was able to circumvent the adverse effects of oral CHQ delivery on blood glucose homeostasis and renal function. We conclude that the pectin-CHQ matrix patch has the potential avert the adverse effects that are associated with oral administration of CHQ.

REFERENCES

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