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A STUDY TO COMPARE THE SYSTEMIC EXPOSURE OF LUMEFANTRINE FOLLOWING A SINGLE DOSE ADMINISTRATION OF COARTEM® (80 MG/480 MG) WITH AND WITHOUT MILK IN HEALTHY VOLUNTEERS

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ABSTRACT

Malaria is still prevalent in many countries and is endemic throughout Cameroon. Despite innovative malaria control tools, morbidity and mortality still remain high, especially in Sub-Saharan Africa. Artemisinin based Combination Therapy (ACT) is the first line treatment for uncomplicated *Plasmodium falciparum* malaria. ACTs combine artemisinin derivatives with a long lasting partner drug. Amongst these ACTs is the combination artemether-lumefantrine which was first branded as Coartem® by Novartis in 1999. Co-administration of artemether–lumefantrine with full cream milk or a fatty diet is recommended to improve the absorption and bioavailability of both drugs. Although research states that a fatty meal or full cream milk will increase the bioavailability of lumefantrine, the local brand of evaporated milk, Peak® regular has no documented evidence to increase the bioavailability of the antimalarial drug lumefantrine. The objective of this study was to determine the safety and tolerability profiles of healthy volunteers to Coartem® (80 mg/480 mg) and to compare the exposure (Cmax, Tmax and AUC $_{(0\text{-}8\ hours)}$) of lumefantrine following single dose administration of Coartem® (80 mg/480 mg) with or without milk in healthy volnteers (HV). A prospective study was carried out from February 2017 to May 2017. In an open-label, two-period crossover study, 14 healthy adult volunteers were randomized to two different sequences to receive a single oral dose of Coartem® (80 mg artemether/480 mg lumefantrine) with 150 ml of water or 150 ml of Peak® regular milk on separate occasions. A washout period of two weeks was allowed between the two treatments. Safety parameters such as blood pressure (BP) and heart rate (HR) were followed up at predose (0 hour), and at postdose (2, 8 and 168 hours). Blood samples were collected at predose (0 hour) and at postdose (0.5, 1, 2, 4, 6 and 8 hours) and assayed using high-performance liquid chromatography with ultraviolet detection. Pharmacokinetic exposure parameters determined were; peak concentrations (Cmax), time to peak concentrations (Tmax) and area under concentration–time curve restricted to 8 hour after single dosing (AUC $_{(0\text{–}8)}$). A paired t- test was used to compare the two treatments and p values <0.05 were considered to be statistically significant. The mean HR and BP prior to dosing (0 hour), post dosing (2 and 8 hours) and 7 days after treatment (168 hours) were found to be within acceptable limits of normal heart rate (i.e. 60-100 bpm) and BP (90-130 mm/Hg) throughout the study. The single dose of Coartem® 80 mg/480 mg was well tolerated by participants as no adverse effects were recorded. Plasma samples were analysed only for the 12 participants who completed the study. Cmax mean values of 2.3 µg/ml following Coartem® + water administration and 6.02 µg/ml following Coartem® + peak® regular milk administration were noted. The mean AUC $_{(0\text{-}8\ hours)}$ values of 9.27 µg/ml.hr and 22.91 µg/ml.hr were observed for the two treatments (i.e. Coartem® + water and Coartem® + peak® regular milk respectively). This difference between the two treatment groups was statistically significant with a p-value < 0.012. This study revealed that the single dose administration of the antimalarial drug Coartem® 80 mg/480 mg was well tolerated by healthy volunteers. The bioavailability of lumefantrine increased by 3 fold with respect to Cmax and AUC $_{(0\text{-}8\ hours)}$ when administered with the local brand peak® regular milk compared to administration with water.

KEYWORDS: Malaria, Lumefantrine, Bioavailability, Milk, High fatty diet.

INTRODUCTION

Malaria is one of the leading cause of morbidity and mortality in developing countries, and remains a major health problem in endemic regions.[1-3] According to the World Health Organization (WHO) in 2016, 3.2 billion people were at risk of malaria; 91 countries and territories had ongoing malaria transmission. Malaria caused 212 million clinical episodes, and 429,000 deaths.[2,4-5] In Cameroon, according to WHO data published in May 2014, malaria deaths reached 12,064 or 5.57 % of total deaths. Malaria was ranked 9th of the top 20 causes of death in Cameroon.[1,6-9]

Generally, artemisinin based combination therapy (ACT) is recommended by WHO as first line treatment for uncomplicated *Plasmodium falciparum* malaria.[4,5,10,11] Due to the short elimination half-life (approximately 2 hours[4,12] of artemisinins, recommendations for the treatment of malaria require the use of artemisinins with partner drugs like lumefantrine (LR) which has a long elimination half-life of approximately 2-3 days in healthy volunteers and 4-6 days in patients.[4,13-15] The role of the artemisinin compound is to reduce the main parasite load during the first 3days of treatment while the role of the partner drug is to eliminate the remaining parasites.

Amongst these ACTs is the combination artemether-lumefantrine (AL) which was first branded as Coartem® by Novartis in 1999. AL is well tolerated, highly effective[6,11,16] and is now becoming the most recommended first-line treatment for uncomplicated falciparum malaria in most African countries.[4,5,17] A six-dose regimen taken over 3 days has excellent efficiency against sensitive and multidrug-resistant *Plasmodium falciparum* malaria.[18] Artemether is a semisynthetic chiral acetal derivative of artemisinin while lumefantrine is a racemic mixture of a synthetic fluorine derivative which interferes with heme degradation.[19-21] Artemether has a fast absorption rate and its major metabolite dihydroartemisinin (DHA) is formed rapidly and has a similar clearance pattern to artemether.

Food intake (especially dietary fat) has been reported to significantly enhance the bioavailability of both artemether and lumefantrine, an effect which is more apparent for the highly lipophilic lumefantrine.[9] Lumefantrine absorption increased 16-fold while that of artemether increased by only twofold when given with a high-fat meal.[4,22,23] This study was designed to investigate the effect of a local brand of readily available milk on the bioavailability (Cmax, Tmax and AUC) of LR.

METHODOLOGY

This study was carried out at the Faith-Based Medical Services (FAMES) clinic at Nouvelle Route Carrossel, Yaounde, Cameroon. The bioanalysis of plasma samples was carried out at National Laboratory for drug quality control (LANACOME). The Study duration was between February and May 2017 for the clinical and the analytical phases.

This study was a prospective, open label, single center, randomized, balanced, single dose, two treatments (fed versus fasting), two periods, two sequence crossover design.

Included in the study were healthy adult volunteers aged between 21 to 45 years who have consented to participate. Excluded from the study were; malaria positive volunteers, pre-existing impairment of liver or renal function, use of inducers or inhibitors of CYP3A4 in at least a month prior to enrolment, pregnant women and participants who did not consume alcohol, caffeine, herbal medicines, grape fruits or grape fruit juice during the study.

Ethical considerations

Ethical clearance was obtained from the IRB of the Faculty of Medicine and Biomedical Sciences of the University of Yaounde I, and the Cameroon National Ethics Committee for studies in human subjects (CNERSH).

Participant Invitation and Selection

The study was advertised in Yaounde and interested healthy volunteers (HV) were requested to turn up on an assigned date at the clinical unit for information dissemination. Volunteers were made to understand that participation in the study was by free will and anyone feeling uncomfortable after the study was explained and were free not to participate. HV meeting the inclusion criteria and expressing comprehension agreeing to take part in the study were requested to read through and sign the consent form before enrolling them into the study.

Informed consent forms written in French and English were available for participants to choose depending on the linguistic preference of the respondent. The aim and benefits of the study was well outlined to all volunteers.

Information and Consent

All volunteers were provided written informed consent to participate in the study prior to being screened. The participant information sheet detailed the procedures involved in the study (aims, methodology, potential risks and anticipated benefits) and the investigator explained these to all participants. The volunteers signed the consent form to indicate that they agree to the terms of the study and willingly volunteered to participate in the study. The original copy of the informed consent form was kept in a confidential file with the investigator.

Withdrawal of Participants from the Study

HV were free to withdraw from the study at any time without giving a reason. The investigator could also withdraw HV from the study if they deemed it appropriate for safety or ethical reasons or if it was

considered to be detrimental to the well-being of the patient. Participants who withdrew underwent a final evaluation.

Full documentation was made of any withdrawals that occurred during the study in the case report form (CRF). The Investigator documented the date and time of the withdrawal and results of any assessments made at this time.

Source of Coartem® 80 mg/480 mg and Milk
Coartem® 80 mg/480 mg was bought from a local community pharmacy and stored at the the clinic ready for use. Peak® milk was purchased from a local supermarket.

Blood Collection
In both sequences, blood samples of 2 ml were collected using sterile syringes at predose (0 hour) and postdose (0.5, 1, 2, 4, 6 and 8 hours). The blood samples were transferred into citrated tubes. After mixing thoroughly with the anticoagulant, the blood samples where centrifuged at 3000 g for 10 minutes. In the biological fluid, plasma was aliquoted and transferred into the corresponding cryotubes. An insulated box with ice was used to transport the blood samples to the laboratory for bio-analyses. The blood samples were frozen (stored at -20°C) until analysis to permit quatification of lumefantrine in each sample.

Randomization Procedure
Participants picked ballot papers with pre-assigned numbers. All those with even numbers were assigned to sequence 1 (AB) and odd numbers assigned to sequence 2 (BA).

Treatment Allocation
Treatment A – 150 ml Peak® regular milk + Coartem® (80 mg/480 mg).
Treatment B – 150 ml mineral water + Coartem® (80 mg/480 mg).

Crossover Design
Each subject in group one received 150 ml of Peak® regular milk in period 1 and after a washout period of two weeks, they received water (period 2). Subjects in group 2 received water in period 1 and after a washout period of two weeks; they received Peak® milk regular each (period 2).

Subjects returned to the clinic 7 days after the last period for follow up. All subjects who participated in one period of the study were followed up.

Clinical Procedure
Screening Day
Two screening days were held. At the end of the two screening days, 20 participants signed the informed consent forms and 19 enrolled into the study.

Periods 1 and 2
Upon arrival of the participants for period one, pretreatment (malaria test, pregnancy test and blood sugar levels to ensure that they all respected the overnight fast) and safety measures (blood pressure and heart rate) were taken. Catheters were placed on the participants by the nurses. Blood samples were obtained at predose (0 hour). Participants were given the assigned treatment (water or milk) and a tablet of coartem®, the dosing time was recorded; a mouth check was performed to ensure that the tablet was swallowed. Further blood samples were obtained at predetermined postdose times (0.5, 1, 2, 4, 6 and 8 hours). BP and HR were measured post-dose at 2 hours and 8 hours. Most of the participants remained ambulatory from time of dosing either using their laptops or discussing among themselves.

Participants were allowed to drink water one hour after dosing and were fed with a standard sandwich (eggs + sausage) four hours after dosing (after the blood sample collection as it coincided).

Blood collected was taken to the laboratory and centrifuged at 3000 g for 10 minutes. Each centrifuged sample was aliquoted into its corresponding cryotube and kept frozen at -20˚C.

Safety Variables and Tolerability profiles
The safety parameters monitored included: Heart rate (HR) and Blood pressure (BP).

Participant BP was measured using a sphygmomanometer after participants were well rested. HR was also measured using a sphygmomanometer for all participants. Participants were asked how they felt at random times during the study while in the clinic. They were told to report all side effects that featured and any feeling of discomfort.

The overall clinical procedure is seen in figure 1.

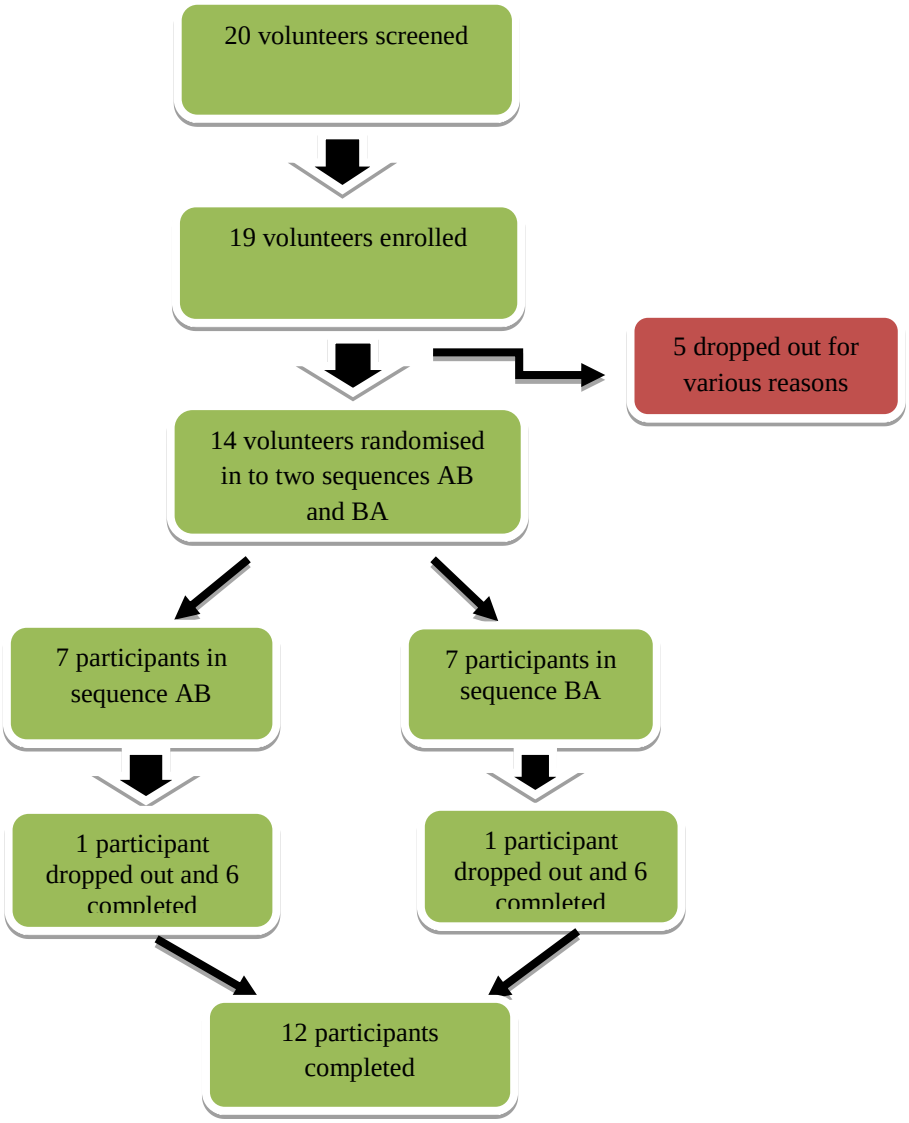

Figure 1: Summary of clinical procedure.

Analytical Procedure

The quantification of lumefantrine in human plasma was performed using a high performance liquid chromatography (HPLC) system for the quantification of lumefantrine in human plasma.[9] A calibration curve was obtained from standard preparations of lumefantrine by linear regression of the peak area of the analyte to the internal standard (Y-axis) versus the nominal concentrations (X-axis). The internal standard was halofantrine.

The HPLC system consisted of a pump, an auto-sampler, an UV-VIS diode array detector (DAD) from Agilent Germany. System management and data acquisition were performed by the Agilent Chemstation software. The chromatographic conditions are shown in table 1 below.

Table 1: Chromatographic conditions.

	Composition
Mobile phase	Solvent A: 0.01% TFA + 0.1M ammonium acetate Solvent B: 0.01% TFA + acetonitrile
Mode	Gradient: 0–2 min (50% B), 9–15 min (98% B) and 15.5–20 min (50% B)
Flow rate	1 ml/min
Injection volume	25 microlitres
Column	C16 3 micrometer 120A 4.6micrometer*150mm
Detection	300 nm
Run time	20 mins

Pharmacokinetic Data analysis

Data from the twelve participants who completed the study was included in all analyses.

The following exposure measures and pharmacokinetic parameters were obtained from the resulting concentration-time curves of the study; Maximum concentration C_{max} observed from data, time ti maximum concentration T_{max} observed from data, Area Under the Curve (AUC_{0-8}) calculated using a linear scale.

Standard T-tests was performed on the mean Cmax and AUC to determine differences between treatments. Significance level was set at $p<0.05$.

RESULTS

Safety Profiles

The mean HR predose and post dose profiles are shown in Figure 2. All participants were observed to remain within the normal limits of HR (60-100 bpm) for both treatments (Figure 2 and 3).

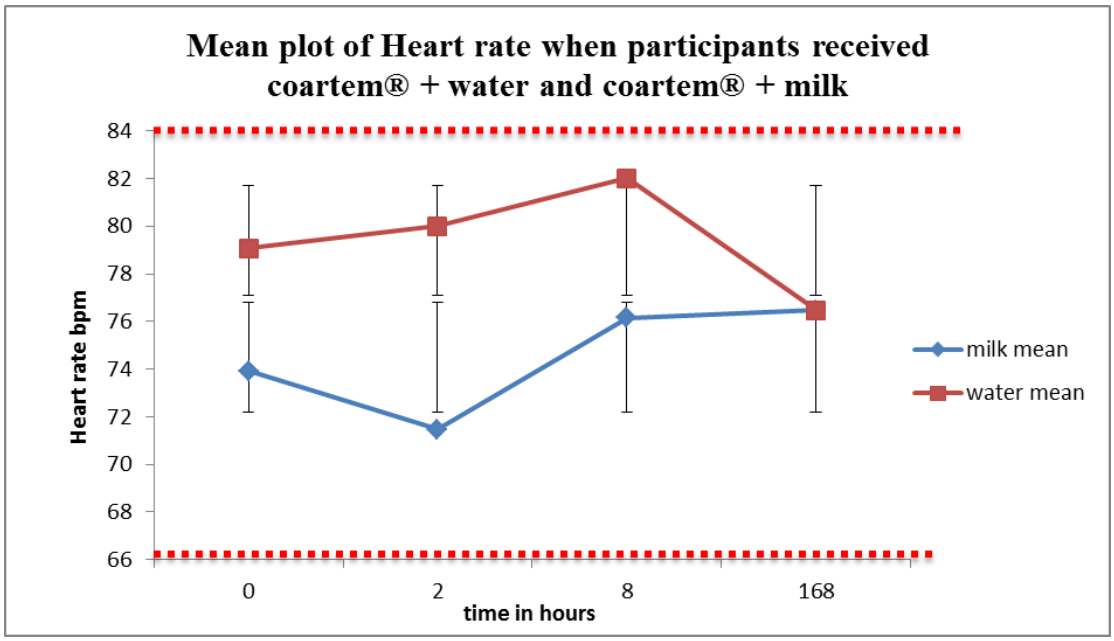

Figure 2: Mean plot of heart rate with water and milk.

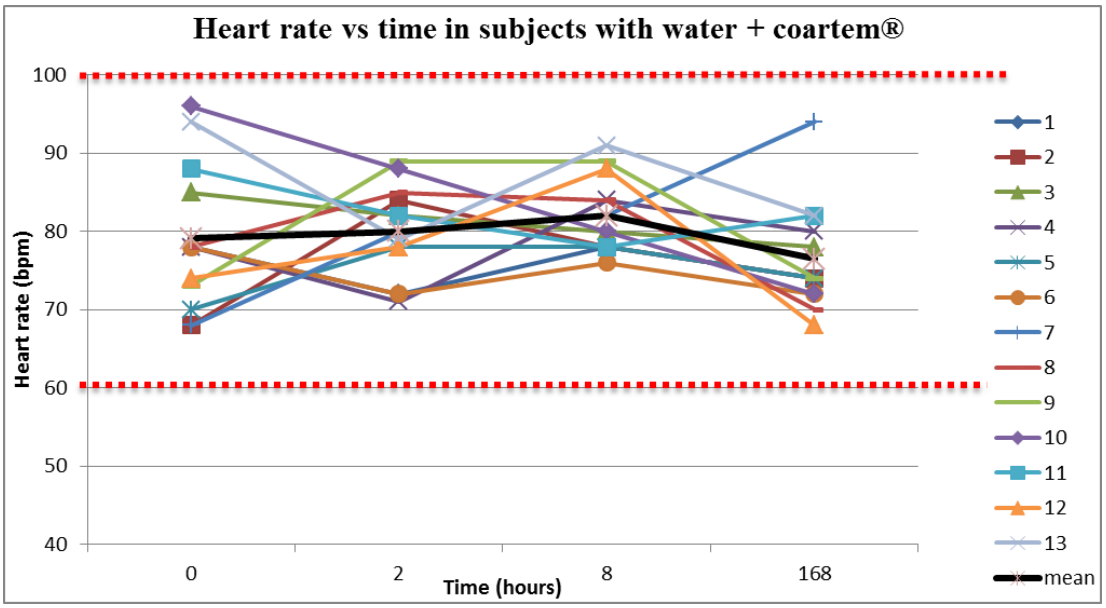

Figure 3: Heart rate versus time in subjects with water + coartem®, KEY 1-13 represents each participants.

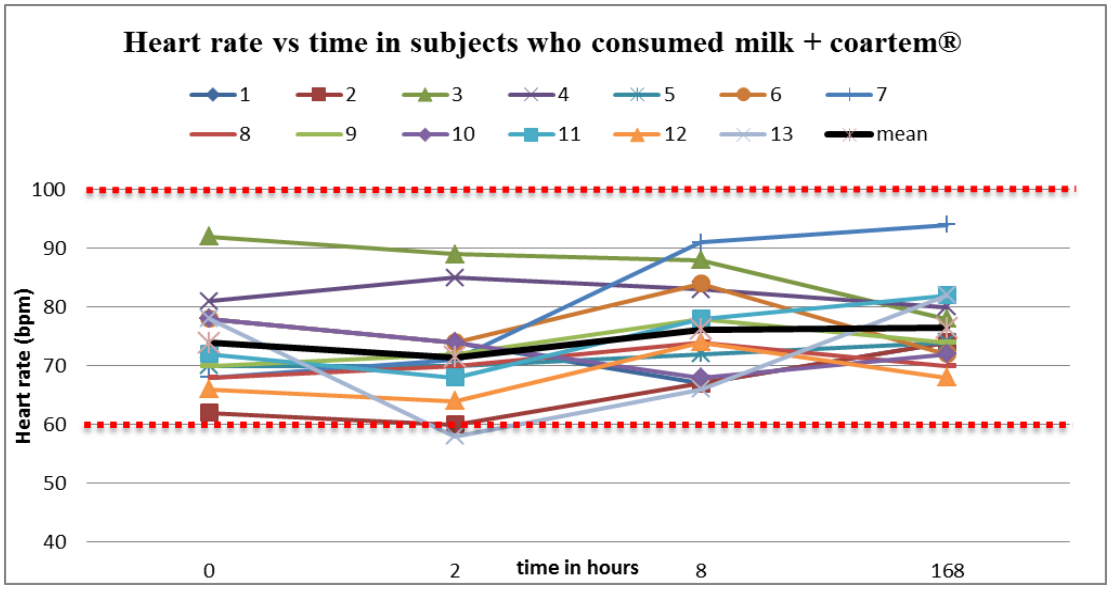

Figure 4: Heart rate versus time in participants with milk+coartem®, KEY 1-13 represents each participant.

A comparison of the mean systolic BP when participants received coartem® with milk to when they received coartem® with water was plotted (Figure 5). The mean systolic BP was between 90-130 mm/Hg with all participants falling within normal/acceptable limits (Figure 5).

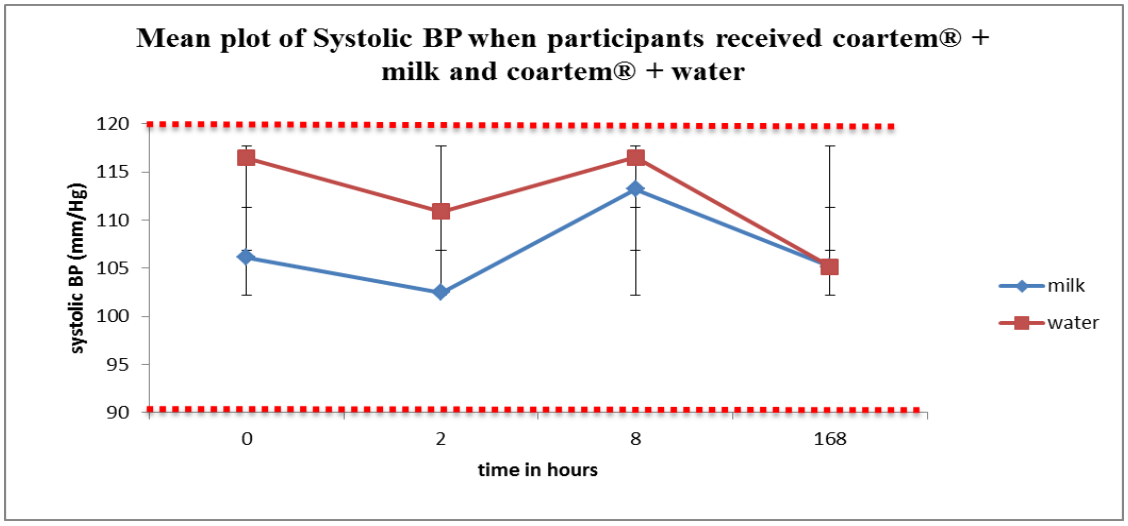

Figure 5: Mean plot of systolic blood pressure when following both treatments.

The mean and standard deviation of systolic blood pressure which was calculated from data when participants received milk and water was not significant (Table 4).

In addition, individual profiles of BPs when participants received coartem® + water showed that one participant deviated from acceptable BP values and when they received coartem® + milk showed that another participant deviated from acceptable BP values (figure 6 & 7). At follow-up, these participants that deviated were seen to fall within the limits of normal BP.

Table 4: Mean and Standard deviation of systolic blood pressure versus time when participants received milk and water.

Time (hours)	Treatment BP (mm/Hg)	
	Milk+ Coartem®	Water + Coartem®
	Mean±SD	Mean±SD
0	106.15±14.29	116.46±19.22
2	102.46±10.47	110.92±11.53
8	113.23±15.14	116.54±10.80
168	105.15±9.36	105.15±9.36

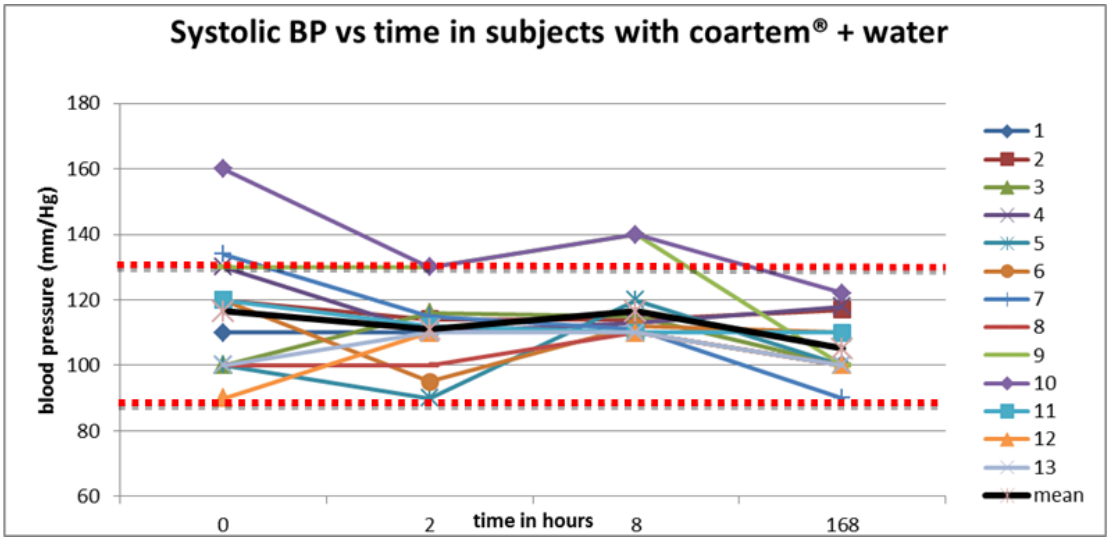

Figure 6: Systolic BP versus time in participants with coartem® + water, KEY 1-13 represents each participants.

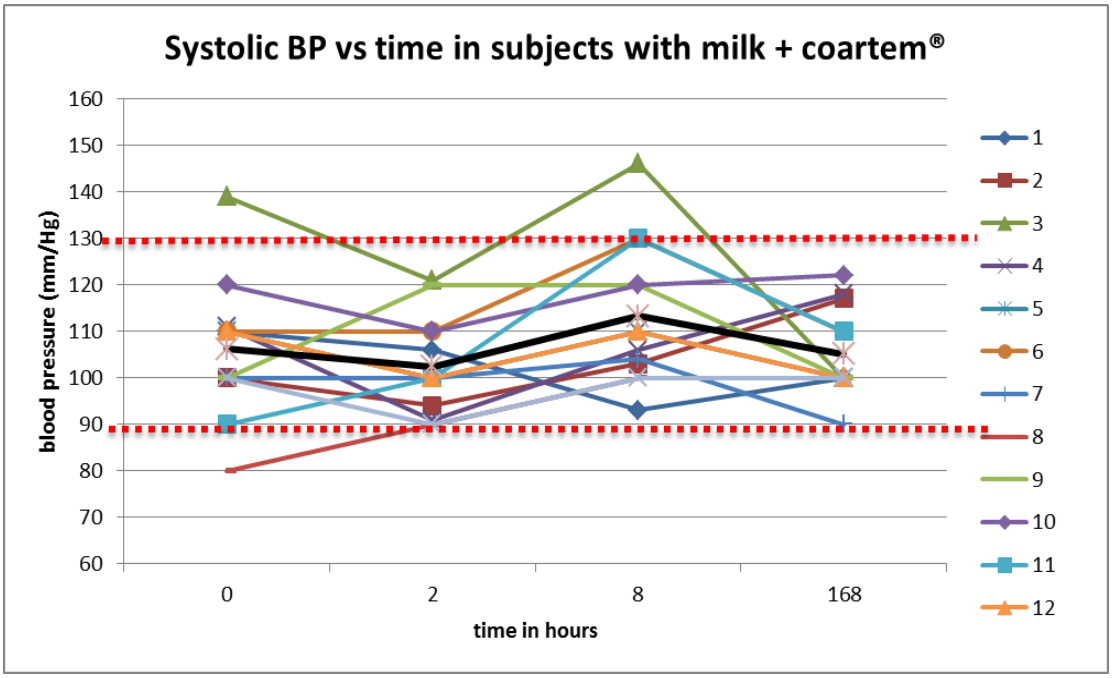

Figure 7: Systolic BP versus time in participants with milk + coartem®, KEY 1-13 represents each participants.

Tolerability: The single dose of coartem 80 mg/480 mg was well tolerated by participants as no adverse effects were reported during the study. Common side effects of coartem® (nausea, vomiting, dizziness) were absent.

Bioanalysis

Identification of Internal Standard (Halofantrine) and the Analyte (lumefantrine)

Halofantrine and lumefantrine were both prepared and run so as to confirm their retention times before running all the samples. A sample of halofantrine alone was prepared and run. This showed halofantrine to be retained at 16 minutes (Fif 8). A mixture of halofantrine and lumefantrine was prepared and run too. This showed halofantrine was retained at 16 minutes and another peak retained at 19 minutes. This other peak was considered to be lumefantrine's peak. The mixture of halofantrine and lumefantrine was run a second time and this confirmed 16 and 19 minutes to be halofantrine's and lumefantrine's retention times respectively as these were the only compounds absorbed at the expected wavelength of 300nm.

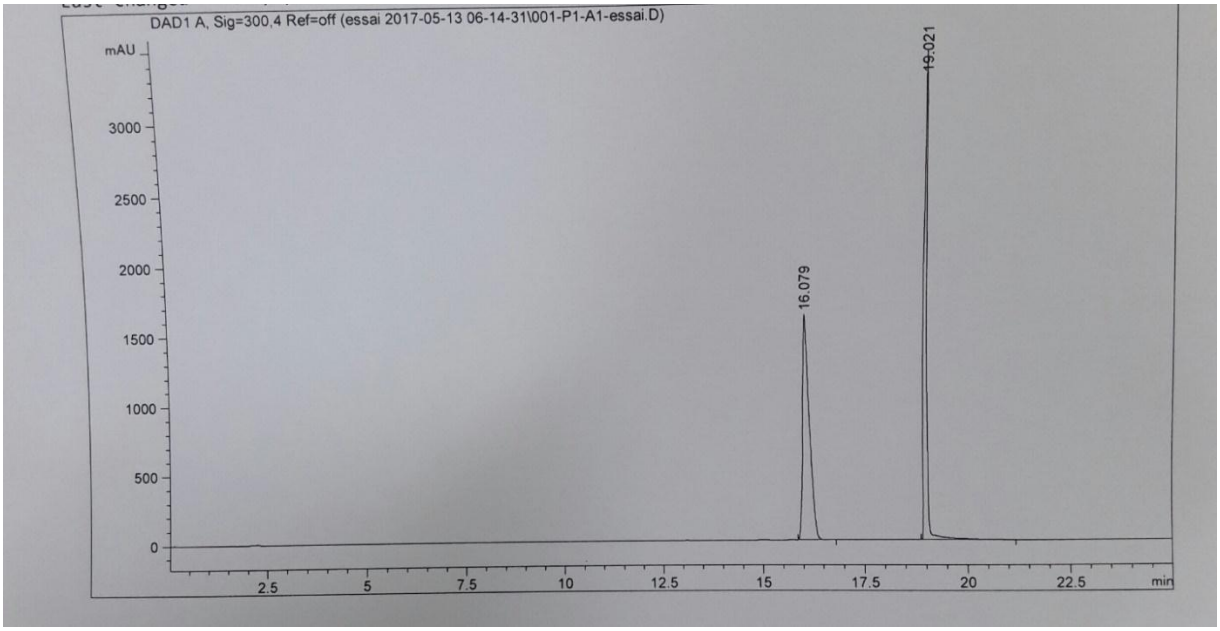

Figure 8: Chromatograms of halofantrine and lumefantrine.

Pharmacokinetic and Data analysis

The following exposure measures and pharmacokinetic parameters were obtained from the resulting concentration-time curves of the study; Maximum concentration (C_{max}) observed from data, Time to maximum concentration (t_{max}) observed from data, Area Under the Curve (AUC_{0-8}) calculated using a linear scale. All predose samples were set to zero where there were quantifiable concentrations of lumefantrine prior to dosing.

The mean plasma concentration versus time profiles of lumefantrine following the administration of Coartem with and without milk is presented in Figure 9 A 3 fold increase in the concentration of lumefantrine was observed with milk compared to water.

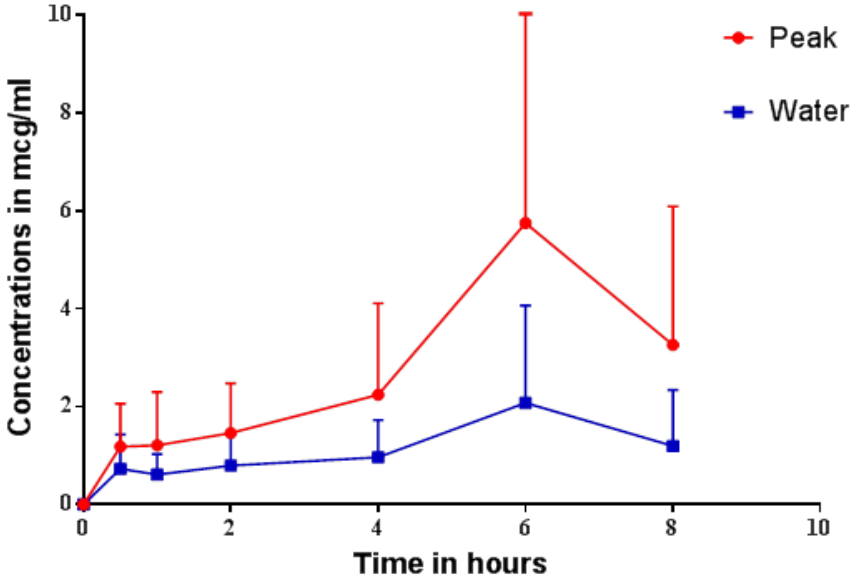

Figure 9: Plot of concentration with time of luminfantrine intake with milk and with water.

There was a statistically significant difference in the Cmax between treatments p-value of 0.003. $AUC_{(0\text{-}8hours)}$ also showed a significant difference between the two treatments (i.e. coartem® + milk and coartem® + water) with p-value of 0.012 (Table 5). Tmax did not show any statistically significant difference between the two treatments.

Table 5: Mean (± SD) pharmacokinetic parameter without predose lumefantrine concentration.

Parameter	Peak	Water	P value (paired t test)
Cmax (mcg/ml)	6.02 ± 5.1	2.3 ± 2.05	0.003
Tmax (hours)	6.5 ± 0.9	6.1 ± 1.83	0.339
$AUC_{(0\text{-}8hours)}$ (mcg/ml/hour)	22.91 ± 17.97	9.27 ± 7.47	0.012

Further analysis using the bioequivalence confidence interval (80-125%) criteria with and without predose concentrations for Cmax and $AUC_{(0\text{-}8\ hours)}$ showed that the two treatments (water + coartem® and peak® + coartem®) are non-bioequivalent (Table 6). This shows that administration with milk has a larger bioavailability than when administered with water.

Table 6: Mean ratio without predose concentration.

Parameter	Mean Ratio (Peak/water)	90% CI
Cmax (mcg/ml)	376.8 %	[268.9 – 498.6]
Tmax (hours)	0.42	P = 0.37
$AUC_{(0\text{-}8hours)}$ (mcg/ml/hour)	288.2 %	[233.8 – 337.7]

The two way analysis of variance (ANOVA) was also used to give additional information on the treatment, the period and the sequence effect (Table 7). This showed a period effect with no treatment nor sequence effects.

Table 7: ANOVA for analysis with predose and without predose concentration respectively.

Parameter	ANOVA (p-value)			90 % CIs (%)
	Treatement	Period	Sequence	
$AUC_{(0\text{-}8hours)}$	0.981	< 0.0001	0.369	[231.5 – 335.31]
Cmax	0.573	0.0002	0.358	[271.8 – 498.8]
$AUC_{(0\text{-}8hours)}$	0.924	< 0.0001	0.385	[233.8 – 337.7]
Cmax	0.519	0.0006	0.390	[268.9 – 498.6]

DISCUSSION

The efficacy of antimalarial treatment in uncomplicated *Plasmodium falciparum* malaria depends on sufficient oral absorption of the antimalarial drug. As a result, a good exposure of the drug within the therapeutic concentrations will ensure eradication of the blood infection. Poor absorption of the drug will lead to low therapeutic concentrations in the body and this is responsible for many cases of treatment failure. Lumefantrine is lipohilic and like many other lipophilic antimalarial drugs, it shows very variable absorption.[24-26] Increased systemic exposure of drugs with food is often seen for lipophilic drugs and is attributable to improved solubilization due to higher bile salt and lipid concentration.

One of the aims of this study was to assess the safety and tolerability profiles of the participants to the combination AL. In this study, the single oral dose of Coartem® was generally well tolerated as no adverse effects were recorded and safe as participants maintained a normal BP and HR. This is similar to findings by Lefevre et al., in 2001[4] where in a study with 16 healthy volunteers receiving a single dose of AL (80 mg/480 mg) no adverse effects were reported.[2,27-30]

This was however different from that reported by Mulenga et al.,[31] in whose study 397 patients treated with coartem® (20 mg/120 mg), 6 patients reported adverse effects (precisely 5 patients having mild adverse effects and one patient presenting serious adverse effects) with the development of a rash considered as the most severe adverse effect of AL.[32] This difference may be partly explained by the use of healthy volunteers and a single dose of coartem® 80 mg/480 mg in our study meanwhile Mulenga et al., used malaria victims wherein 6 doses of coartem® 20 mg/120 mg were administered.

In a study carried out by Djimde et al.,[33] and Lefevre et al.,[4,34-35] the combination AL was generally reported and concluded to have excellent tolerability as was the case in our study.

The bioanalytical results from this study showed concentrations at the pre-dose sampling time. These results consistent in all of the subjects and in both study periods. These small concentrations found in the predose samples were treated in the data analysis by setting the concentrations to 0. This approach was used because there was no significant difference in data sets where predose concentrations were used in analysis.

Secondly, according to the European Medicines Agency (EMA) guideline on bioequivalence (BE) report, if the predose concentration is greater than 5 % of the Cmax value, the subject should be removed from the analysis.[2,39-41] The data can be modulated with the

predose concentrations if all the predose values are lower than 5 % of the respective Cmax.[13,42] In this study, two participants were seen to have predose concentration slightly greater than 5 % of the Cmax value. Data was generated for all 12 participants as the deviation seen was slightly greater than 5%.

Findings from the study showed that the relative oral bioavailability (rate and extent of absorption) of lumefantrine increased when artemether–lumefantrine was co-administered with a fatty meal than when it was administered with water. This study showed a statistically significant difference with paired t-test between the Cmax of Lumefantrine with milk compared to that of water with a p-value < 0.05. This was similar to a study carried out in 1999 by Lefevre et al[4,43] which showed an increase in the Cmax of lumefantrine when 16 healthy volunteers received food as to when they received water. The same scenario was reported in a study by Mwebaza et al., in 2013 in which 13 healthy volunteers were used.[5,44-46]

Furthermore, in this study, Tmax did not show a statistically significant difference between the two treatments. This was similar to results reported by Lefevre et al., in their study in 1999[4,9,47] where Tmax was seen to be 6 hours for both groups (with water and food).

However, Tmax was found to be statistically significant when lumefantrine was administered with milk to when it was administered with water in a study carried out by Mwebaza et al., in 2013.[16,27,48] This difference could partly be explained by the difference in the environmental conditions and the inter subject variability experienced by the volunteers.

In our study, the AUC $_{(0\text{-}8hours)}$ showed a statiscally significant difference between lumefantrine in coartem® co-administered with milk compared with water. A 3 fold increase in lumefantrine's concentration when administered with milk was reported, this however differed from studies reported by Lefevre et al., in 1999[4,11,50] showing a 16 fold increase in lumefantrine's concentration when administered with a standard fatty meal in 16 healthy volunteers. A study by Mwebaza et al., study in 2013[16,51-52] showed a 14 fold increase in lumefantrine's concentration when administered with 200 ml of milk in 13 healthy volunteers. These disparities could partly be explained by the fact that Lefevre et al,[4] used a standard high fat diet during their study and Mwebaza et al.,[16] used 200 ml of whole milk containing approximately 6.8 g of fat. The differences in lumefantrine bioavailability between these two studies and ours could be explained by the remarkably low fat content of the brand of milk, peak® used in our study.

CONCLUSION

At the end of this study, we can draw the following conclusions: The single dose of Coartem® 80 mg/480 mg which is the combination of the two active antimalarial pharmaceutical ingredients artemether and lumefantrine, was well tolerated and safe for use.

The study showed that when Coartem® 80 mg/480 mg was co-administered with 150 ml of the local brand Peak® regular milk, there was a 3-fold increase in the Cmax and partial AUC (0-8) of the plasma concentration of lumefantrine compared to when co-administered with water.

Tmax did not show any significant difference when Coartem® was co-administered with 150 ml of water or with 150 ml of Peak® regular milk..

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