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Alteration in Serum Lipid Profile following Separate Administration of Anti-Malarial Drugs (Coartem and Chloroquine): A Comparative Study

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ABSTRACT

Over the years, various anti-malarial drugs have been introduced to combat malaria parasite, with coartem and chloroquine being widely prescribed in hospitals in Nigeria. This study was designed to investigate the effect of coartem and chloroquine separately administered, on serum lipid profile which gives important preliminary information on possible adverse effect on cardiovascular health. Thirty male Wistar rats weighing 180 – 200 g were used for this study. After 7 days of acclimatization, the animals were divided into 2 batches (n = 15); thus A (3 days treatment) and B (7 days treatment). Each batch was further divided into 3 groups (n = 5) as follows; control, coartem treated group and chloroquine treated group. The treatment lasted for 3 days and 7 days for batch A and B respectively. The drugs were orally administered and all animals had access to food and water ad libitum. Blood was collected via cardiac puncture for lipid profile analysis. Results showed that serum total cholesterol was significantly (P<0.05) higher in the 7 days coartem treated group, compared with the 3 days coartem treated group. Low density lipoprotein and atherogenic index were significantly (P<0.05) increased in the 7 days coartem treated and chloroquine treated group, compared with the 3 days coartem treated and chloroquine treated group respectively. We therefore conclude that administration of either coartem or chloroquine for 3 days is safe, but can predispose to cardiovascular diseases secondary to altered serum lipid profile if administered for 7 days.

Keywords: Anti-malarial drug, chloroquine, cholesterol, coartem, lipid profile, serum.

INTRODUCTION

Malaria, a disease caused by a bite from a female *Anopheles* mosquito carrying *Plasmodium falciparum* is a life threatening disease, with nearly half of the world's population being vulnerable [1]. The estimated annual deaths secondary to malaria infection has been fixed at 2 – 3 million [2]. The prevalence of malaria infection depends widely on the surrounding environment, being highest in tropical areas and lowest in non – tropical areas. In Nigeria, there is a good combination of adequate rainfall, humidity and temperature. This allows for breeding and survival of anopheles mosquitoes which is the main carrier of malaria parasite. Out of the over 100 species of malaria parasite, four species (*P. falciparum*, *P. vivax*, *P. malariae* and *P. oval*) of *Plasmodium* have been found to infect humans. Of the four species that infect humans, *Plasmodium falciparum* has been reported to be responsible for most of the severity and deaths attributed to malaria [2]. Malaria infection usually present symptoms like fever, headache, nausea and vomiting. The World Health Organization (WHO) had estimated that malaria killed between 660,000 and 1.2 million people in 2010, while there were 219 million documented cases of the infection, many of whom were Africans [3].

A number of anti – malarial drugs have been introduced for treatment of malaria infection, with chloroquine being one of the oldest therapies, while coartem (artemether/lumefantrine) is more recent. Chloroquine was used for prevention and treatment of malaria, until the 1950s when the malaria parasite developed chloroquine resistance [4, 5]. Coartem is an anti-malarial drug indicated for treatment of acute, uncomplicated malaria infections caused by *Plasmodium falciparum* [6, 7]. Coartem has been shown to be effective in treatment of malaria in geographical regions where resistance to chloroquine has been reported [8]. Artemisinin (a component of coartem) and its derivatives are rapidly eliminated and have been shown to produce rapid resolution of symptoms as well as rapid clearance of parasitaemia [9].

Most anti – malarial drugs are being abused in the course of preventing or treating malaria infections, especially among individuals who indulge in self – medication rather than seeing a qualified medical personnel. A number of side effects accompany drug abuse, anti – malaria drugs inclusive. Previous studies had reported that administration of coartem for up to 7 days predisposes to hypernatremia and hypokalemia [10]. Considering the wide spread prevalence of malaria infection, and following the promoted use of anti – malarial drugs for prevention and treatment of malaria infection, it became necessary to ascertain the effect of anti – malarial drugs (coartem and chloroquine) on serum lipid profile which is an important biochemical index that gives information on whether or not an individual is likely to develop cardiovascular disease.

MATERIALS AND METHODS

Drug Preparation

The present study employed coartem and chloroquine (anti-malarial drugs) which are widely prescribed and abused as well. Coartem (Novartis Pharmaceuticals Corporation, Basel, Switzerland) and chloroquine (Novartis Pharmaceutics, USA) were purchased from UNIPERVIT Pharmacy, Calabar, Nigeria. One tablet of coartem (140 mg) was dissolved in 10 ml of distil water (concentration of 14 mg/ml), while 1 tablet of chloroquine (150 mg) was dissolved in 10 ml of distil water (concentration of 15 mg/ml). The drugs were usually dissolved and prepared at the point of administration and same was given to the animals immediately.

Experimental Animals and Protocol

Thirty male Wistar rats weighing 180 – 200 g were obtained from the animal house of the Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria. The animals were allowed to acclimatize for 7 days in well-ventilated cages. They were exposed to normal temperature and 12/12 hours light/dark cycle. All animals had access to standard rodent chow (TOP Feed Ltd., Sapele, Nigeria) and tap water *ad libitum.* After habituation period, the animals were randomly divided into 2 batches (A and B; n = 15). Each batch was further divided into 3 groups (n = 5) as follows;

Group 1 – Control
Group 2 – Coartem treated group
Group 3 – Chloroquine (CQ) treated group

Drug Administration

After seven days of acclimatization, the drugs were orally administered to the test groups at a dose of 15.4 mg/kg and 8.75 mg/kg for coartem and chloroquine respectively. The second dose of coartem for day 1 was administered 8 hours after the first dose. The drugs were subsequently administered twice daily. Administration was facilitated by the use of a syringe and orogastric tube. Drug administration for batch A animals lasted for 3 days, after which the animals were sacrificed, while that of batch B lasted for 7 days. All procedures in this study were in line with approved guidelines of the local ethics committee of the College of Medical Sciences, University of Calabar, Nigeria, and were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Sample Collection

At the end of the drug administration period for each batch, the animals were anesthetized using chloroform anaesthesia. Blood samples were then collected via cardiac puncture using a 5 ml syringe and needle. Samples were collected into plain labelled sample bottles and centrifuged at 3000 rpm for 10 minutes to separate and collect serum for lipid profile analysis.

Measurement of Serum Lipid Concentration

Total cholesterol (TC) concentration was determined by the enzymatic colorimetric test kit method of Sieldel et al., [11]. Serum triglyceride (TG) concentration in the samples was measured by method of Negele et al., [12]. High density lipoprotein cholesterol (HDL-c) was determined by method of Siedel et al., [11] for total – cholesterol estimation, while VLDL-c concentration was obtained by dividing the serum TG concentration by 5. This factor of 5

is based on the fact that in fasting subjects with triglyceride concentration of 400 mg/dl, the ratio of VLDL to total plasma triglyceride is fixed at 1:5, thus:

$$\text{VLDL-c (mg/dl)} = \frac{\text{Triglyceride (TG)}}{5}$$

Low density lipoprotein cholesterol (LDL-c) was measured by the Friedewald's [13] relationship. LDL-cholesterol was obtained by subtracting HDL-c and VLDL-c from TC as shown below;

$$\text{LDL-c} = \text{TC} - (\text{HDL-c} + \text{VLDL-c})$$

Atherogenic index was obtained using the formula [14]:

$$\text{Atherogenic Index (AI)} = \frac{\text{LDL-c}}{\text{HDL-c}}$$

Statistical Analysis

All results are presented as mean ± SEM. One way analysis of variance (ANOVA) was used to analyse data within the same batch of animals, while Student's t – test was used to analyse differences between batch A and B. Statistically significant difference was employed ate $P < 0.05$. Statistical software, SPSS version 17.0 and Microsoft excel (2010 version) were used for data analysis.

RESULTS

Total Cholesterol

The mean total cholesterol concentration for control, coartem treated group and chloroquine treated group was 186.41 ± 12.51, 192.15 ± 8.57 and 187.32 ± 19.12 mg/dL respectively, for day 3. Mean TC for day 7 was 207.54 ± 17.66, 228.96 ± 10.23 and 230.62 ± 10.58 mg/dL for control, coartem treated and chloroquine treated group respectively. There was no significant difference in mean total cholesterol in the different groups within the two batches A and B (Figure 1). Total cholesterol was significantly ($P<0.05$) higher in the 7 days coartem treated group, compared with the 3 days coartem treated group, (Table 1).

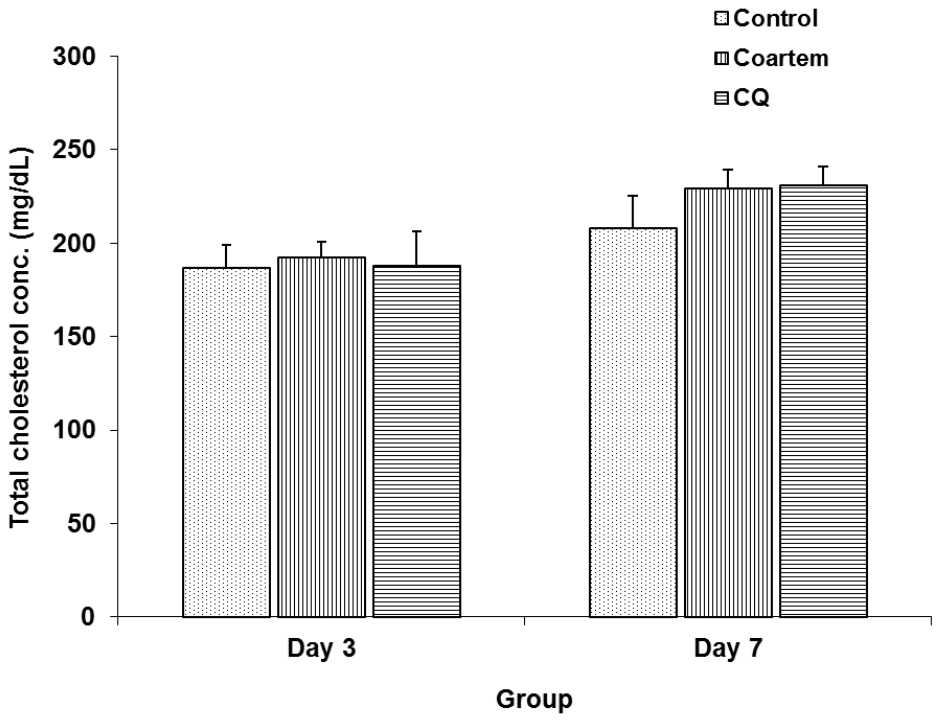

Figure 1: Comparison of total cholesterol (TC) concentration in the different experimental groups at days 3 and 7.
Values are mean ±SEM, n = 5.

Serum Triglyceride Concentration

The mean serum TG concentration for day 3 was 157.59 ± 31.87, 107.54 ± 5.63 and 123.15 ± 21.64 mg/dL, for control, coartem treated and chloroquine treated group respectively. Mean serum TG concentration for day 7 was 121.01 ± 11.44, 121.57 ± 13.46 and 109.11 ± 4.61 mg/dL for control, coartem treated and chloroquine treated group respectively. There was no significant difference in serum TG concentration within the 2 batches – A and B (Figure 2).

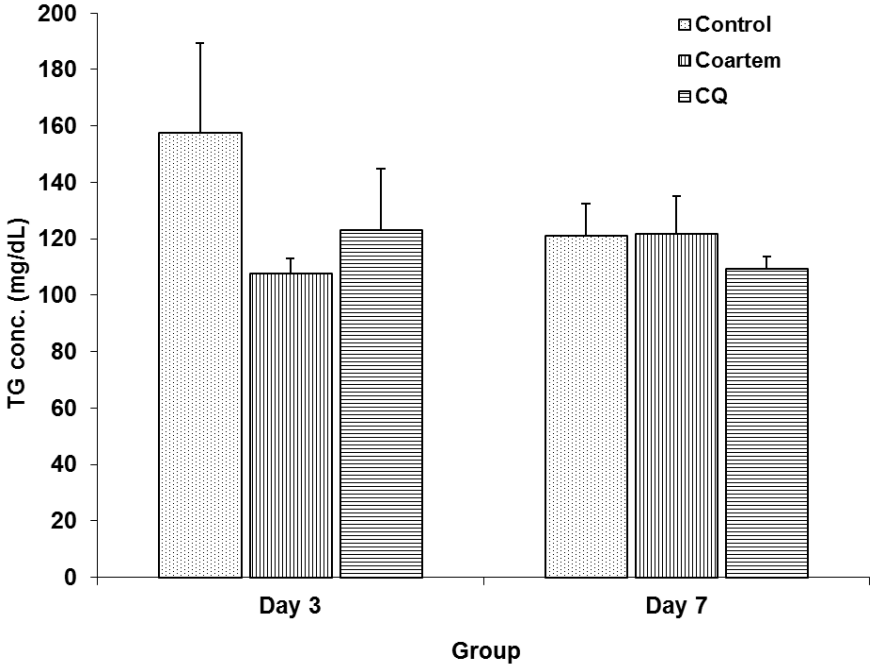

Figure 2: Comparison of triglyceride (TG) concentration in the different experimental groups at days 3 and 7.
Values are mean ±SEM, n = 5.

High Density Lipoprotein (HDL-c) Concentration

The mean concentration of serum HDL-c in the control, coartem treated and chloroquine treated group was 90.56 ± 0.95, 89.98 ± 2.18 and 89.24 ± 0.66 mg/dL respectively, for day 3. That of day 7 was 89.04 ± 0.52, 88.45 ± 0.85 and 89.06 ± 0.60 mg/dL for control, coartem treated and chloroquine treated group respectively. There was no significant difference in serum HDL-c concentration in the different groups within the batches 2 batches, (Figure 3).

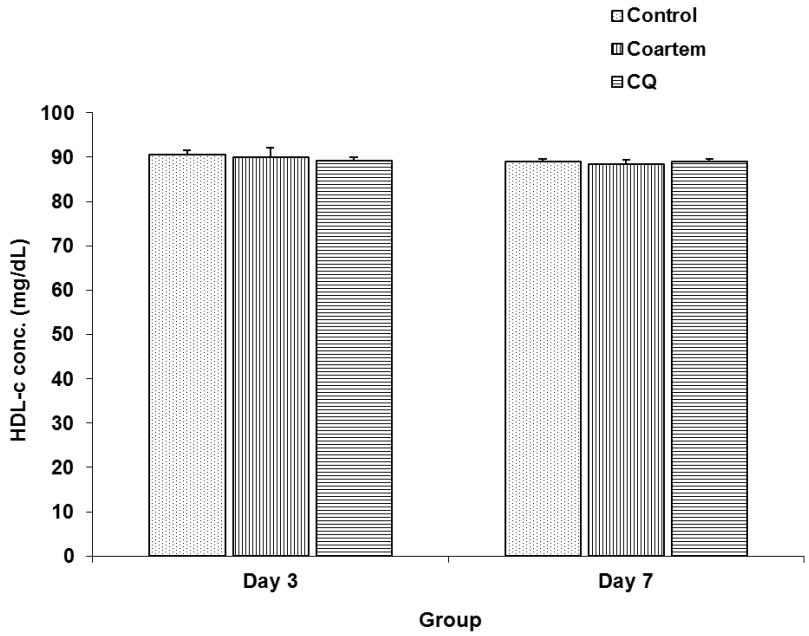

Figure 3: Comparison of high density lipoprotein cholesterol (HDL-c) concentration in the different experimental groups at days 3 and 7.
Values are mean ±SEM, n = 5.

Low Density Lipoprotein (LDL-c) Concentration

Mean LDL-c concentration at day 3 for control, coartem treated and chloroquine treated group was 64.33 ± 11.85, 80.66 ± 10.47 and 73.45 ± 15.33 mg/dL respectively, and that of day 7 was 94.30 ± 16.52, 116.19 ± 10.23 and 119.74 ± 10.56 mg/dL for control, coartem treated and chloroquine treated group respectively. There was no significant difference in serum LDL-c concentration in the different experimental groups within the batches – A and B (Figure 4). Low density lipoprotein concentration was significantly ($P<0.05$) higher in the 7 days coartem treated group, compared with the 3 days coartem treated group. It was also significantly ($P<0.05$) higher in the 7 days chloroquine treated group, compared with the 3 days chloroquine treated group (Table 1).

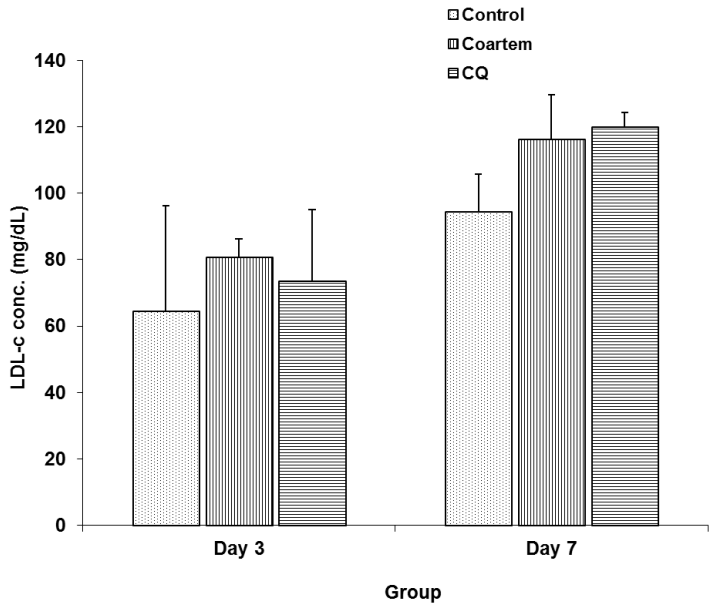

Figure 4: Comparison of low density lipoprotein cholesterol (LDL-c) concentration in the different experimental groups at days 3 and 7. *Values are mean ±SEM, n = 5.*

Very Low Density Lipoprotein (VLDL-c) Concentration

The concentration of VLDL-C in the control, coartem and chloroquine treated group for day 3 was 31.52 ± 6.37, 21.51 ± 1.13 and 24.63 ± 4.33 mg/dL respectively. VLDL-c concentration for day 7 was 24.20 ± 2.29, 24.31 ± 2.69 and 21.82 ± 0.92 mg/dL for control, coartem and chloroquine treated group respectively. There was no significant difference in VLDL-c concentration in the different experimental groups for day 3 and 7 respectively, (Figure 5).

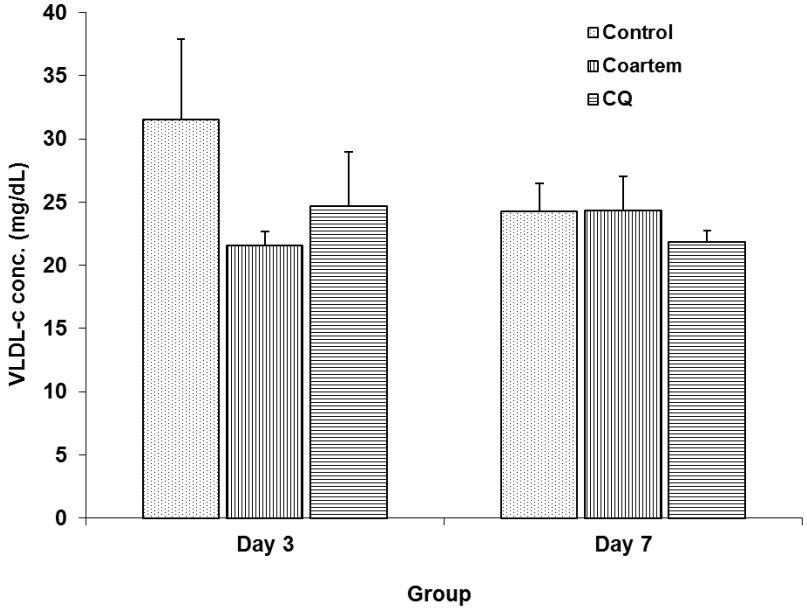

Figure 5: Comparison of very low density lipoprotein cholesterol (VLDL-c) concentration in the different experimental groups at days 3 and 7. *Values are mean ±SEM, n = 5.*

Atherogenic Index (AI)

Atherogenic index for control, coartem and chloroquine treated group was 0.71 ± 0.13, 0.91 ± 0.13 and 0.82 ± 0.17 respectively. There was no significant difference in AI in the different groups studied for day 3 and 7 respectively (Figure 6). Atherogenic index was significantly (P<0.05) higher in the 7 days coartem treated group, compared with the 3 days coartem treated group. It was also significantly (P<0.05) higher in the 7 days chloroquine treated group, compared with the 3 days chloroquine treated group (Table 1).

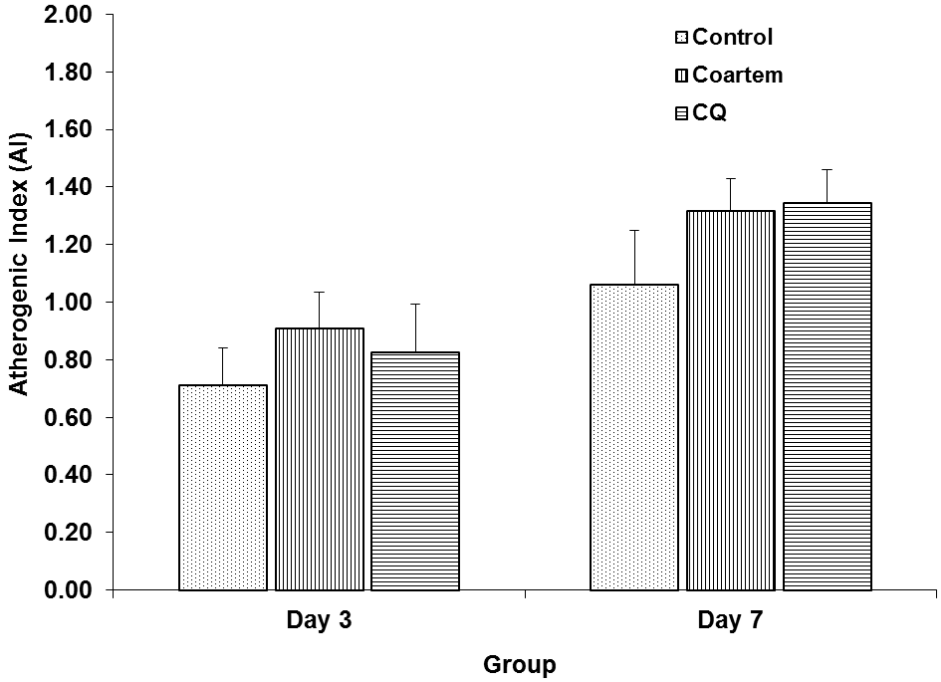

Figure 6: Comparison of atherogenic index (AI) in the different experimental groups at days 3 and 7.
Values are mean ±SEM, n = 5.

Table 1: Effect of duration of treatment with anti-malarial drugs (coartem and chloroquine) on lipid profile

	Day 3			Day 7		
	Control	**Coartem**	**CQ**	**Control**	**Coartem**	**CQ**
TC (mg/dL)	186.41 ±12.51	192.15 ±8.57	187.32 ±19.12	207.54 ±17.66	228.96 ±10.23*	230.62 ±10.58*
HDL-c (mg/dL)	90.56 ±0.95	89.98 ±2.18	89.24 ±0.66	89.04 ±0.52	88.45 ±0.85	89.06 ±0.60
TG (mg/dL)	157.59 ±31.87	107.54 ±5.63	123.15 ±21.64	121.01 ±11.44	121.57 ±13.46	109.11 ±4.61
LDL-c (mg/dL)	64.33 ±11.85	80.66 ±10.47	73.45 ±15.35	94.30 ±16.52	116.19 ±10.23*	119.74 ±10.56*
VLDL-c (mg/dL)	31.52 ±6.37	21.51 ±1.13	24.63 ±4.33	24.20 ±2.29	24.31 ±2.69	21.82 ±0.92
AI	0.71 ±0.13	0.91 ±0.13	0.82 ±0.17	1.06 ±0.19	1.31* ±0.12	1.34* ±0.11

*Values are expressed as mean ±SEM, n = 5. *P<0.05 vs day 3.*

DISCUSSION

Lipid measurements are integral components of risk prediction in the primary prevention of cardiovascular disease and management of therapy in the primary and secondary prevention of coronary heart disease (CHD). Raised total cholesterol, LDL-c and reduced HDL-c levels have all been shown to be strong independent risk factors for CHD in numerous cross-sectional, prospective and intervention trials. Kannel had shown in his study that HDL-c is the single lipid/lipoprotein predictor of cardiovascular and CHD mortality [15, 16], while LDL-c predicts CHD only marginally better than total cholesterol. Since the use of chloroquine and coartem for prevention and treatment of malaria infection in Nigeria have been widely promoted over the years, this study was designed to ascertain the impact of coartem or chloroquine administration on lipid profile. Separate administration of coartem and chloroquine did not significantly affect serum lipid profile in the groups administered, compared with control. However, there was a decrease in TG and VLDL-c in the coartem and chloroquine treated group, compared with

control. Coartem and chloroquine treated groups also had an increased LDL-c and atherogenic index, compared with control.

Separate administration of coartem and chloroquine for 7 days significantly increased TC concentration compared with the 3 days treated groups. Results also showed that neither coartem nor chloroquine had detrimental effects on TG, HDL-c and VLDL-c when administered for 3 days and 7 days respectively. However, LDL-c was significantly ($P<0.05$) increased in the coartem and chloroquine group treated for 7 days, compared with those treated for 3 days (Table 1).

From our results shown in Table 1, administration of coartem or chloroquine for 3 days had no profound effects on the lipid profile, an indication that these drugs when properly administered would not predispose the patients to any form of CHD secondary to a compromised lipid profile, but prolonged intake of the drugs or overdose (7 days treatment) may result in deleterious effects on the body as evidenced in significantly altered TC, LDL-c and atherogenic index.

CONCLUSION

In conclusion, administration of either coartem or chloroquine for 3 days is safe, but can predispose to cardiovascular diseases secondary to altered serum lipid profile if administered for 7 days.

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