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Effects of Chloroquine and Coartem on Haematological Parameters in Rats

Ofem, O. E., [2]Essien, N. M and [3]Okon, U. A.

[1]Department of Physiology, College of Medical Sciences, University of Calabar, Nigeria.
[2]Department of Biochemistry, College of Medical Sciences, University of Calabar, Nigeria.
[3]Department of Physiology, College of Medical Sciences, University of Uyo, Nigeria

ABSTRACT

Coartem has recently replaced chloroquine as the first-line treatment for uncomplicated *Plasmodium falciparum* malaria due to resistance in many regions worldwide. This study was aimed to compare the effect of chloroquine and coartem on haematological parameters in rats. Thirty (30) albino Wistar rats were randomly assigned into 2 batches of 15 rats. Each batch was further divided into 3 groups of 5 rats each. Group 1 was control, groups 2 and 3 received coartem (1.6mg/100g body weight) and chloroquine (0.875mg/100g body weight) orally and once daily. The feeding regimen lasted for 3 and 7 days for batches 1 and 2 respectively. Complete blood count was done using automatic counter. Results revealed that administration of chloroquine and coartem for 3 days did not significantly altered the levels of RBC, Hb, PCV, total & differential WBC, platelet count and platelet indices, but led to significant reductions in MCV and MCH in chloroquine recipient (60.80 ±0.98fL and 19.14 ±0.31pg) compared with control (68.64 ±2.12fL and 20.80 ±0.60pg; $p<0.05$) and coartem (68.16 ±1.73fL and 20.36 ±0.14pg; $p<0.01$) groups respectively. However, administration of these drugs for 7 days caused significant reduction in RBC, Hb and PCV in coartem recipients compared with control ($p<0.05$) and chloroquine ($p<0.01$) groups. RDW was also significantly reduced in chloroquine recipients. In conclusion, administration of coartem and chloroquine at their recommended doses and durations would not pose any deleterious effect on haematological parameters in rats..

Keywords: Blood, antimalaria, chloroquine, coartem, blood, rat.

INTRODUCTION

Malaria is a mosquito borne disease, which has posed a serious health challenge to human. It is an infectious disease caused by a mosquito parasite called *Plasmodium*. The vast majority of deaths by mosquitoes are caused by *P.* f*alciparum*, the other species of malaria parasite are *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* and are less effective in causing threaten to life (WHO, 2006; Cox-Singh, 2008; Fairhust and Wellems, 2010).

The *plasmodium* affects blood cells, malaria symptoms are accompanied by fever, shaking chills, sweating, pains etc which is widely affecting people in the tropic region, tormenting about 400-600 million of people yearly, with over 3-5 million death (Joy *et al.*,

*Address for correspondence:
Email: ofemo2003@yahoo.com
Tel: +2348137026600; +2348055929850

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2003, Snow *et al*., 2005; Mulenga *et al*, 2006; Hay *et al.*, 2010; World Malaria Report, 2010).

To prevent the menace of malaria among the populace, anti-malarial drugs have been produced to either prevent or cure the occurrence of malaria. The earliest drugs which have long been used in the treatment and prevention of malaria were the aminoquinolines of which chloroquine was the mainstay from 1934 (Greenwood, 1995; WHO, 2008; Kraft *et al.*, 2012; White, 2004), but the emergence and spread of resistance in a large population of the African continent had led to the introduction of artemisinin based combination therapy (ACTs) in the 21st century. Zambia was the first African country to adopt ACT treatments in its policy, (Spilanyambe *et al.*, 2008). Among the ACT drugs of choice is coartem, (Nosten and White, 2007; Katzung, 2007).

Coartem (Arthermether + Lumefantrine) is currently the only fixed dose artemisinin based combination therapy prequalified by the WHO for procurement by United Nations agencies for the treatment of acute uncomplicated plasmodium *Falciparum*, (Falade *et* al., 2005; WHO, 2010). Artemether is one of the semi synthetic derivatives of artemisinin, Artemisinin is a natural anti-malarial derived from the Chinese medicinal plant *Artemisia annua*. The artemisinin derivatives are the most effective anti-malarial drugs available today and they have been used with success in areas with multidrug resistant *Plasmodium falciparum* malaria (Adjuik , 2004; Sinclair *et al*., 2009; Kokwaro, 2007; Koram *et al*., 2012). Lumefantrine (also known as benflumetol and CGP 56695 during development) is purely synthetic, (Makenga *et al.*, 2002; Novartis, 2005).

The use of these drugs to treat malaria may be associated with possible side effects on the blood cells, it was therefore the aim of this study to undertake a comparative effect of chloroquine and coartem on haematological parameters (RBC count, PCV, Hb, MCV, MCH, MCHC, RDW, WBC count, Platelets count, platelet indices-MPV, P-LCR, PDW and differential WBC count).

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MATERIALS AND METHODS

Animals

Thirty (30) albino Wistar rats (initially weighing between 120-160g) were obtained from animal house of the Department of Physiology, University of Calabar and housed in cages in the laboratory of the Department of Physiology, College of Medical Sciences, University of Calabar.

The animals were allowed to acclimatize for one week in a well aerated room at room temperature under natural lighting condition of 12 hour light and 12 hour dark cycle. All the animals were handled under standard guidelines for care and use of laboratory animals as promulgated by Canadian Council of Animal Care (2009).

Drugs

Coartem: Coartem manufactured by Beijing Norvatis Pharma LTD, Beijing, China for Norvatis Pharmabarle, Switzerland under license from the PRC was obtained from the Bez Pharmacy, Calabar, Nigeria. One tablet (140mg) of coartem was crushed using a glass mortar and it was dissolved in a total of 10ml of distilled water to give a concentration of 14mg/ml stock. The drug was administered to the animals at a dose of 1.6mg/100g body weight [equivalent to human (70kg) daily dose], i.e. 0.11mL of stock /100g body weight After every administration the remaining drug was poured away and a new one prepared following the next administration.

Chloroquine: Chloroquine was obtained from Turtle Bay Pharmacy, Calabar-Nigeria. One tablet (150mg) was ground to powder and dissolved in a total of 20ml of distilled water to give a stock concentration of 7.5mg/ml. The drug was first administered at a dose of 0.875mg/100g body weight (i.e. 0.12mL of stock/100g body weight) for the first 2 days. On the 3rd day, 4.38mg/kg body weight (i.e. 0.06mL of stock/100g body weight) was administered.

After every administration, the remaining drug was poured out and a new one was prepared following the next administration

Experimental Design

Thirty (30) albino Wistar rats were assigned into 2 batches of 15 rats each. Each batch was divided into 3 groups of 5 rats each and fed thus:

- Group 1 (control) received normal rat chow + water.
- Group 2 (coartem treated): in addition to control diet received coartem treatment orally and once daily,
- Group 3 (chloroquine treated): in addition to the control diet received chloroquine treatment orally and once daily.

Treatment lasted for 3 days and 7 days for batches 1 and 2 respectively.

Collection of Blood Samples

The animals were made unconscious inhaling chloroform anesthesia (3.5% soaked in cotton wool) and blood collected via cardiac puncture (blood was drawn from the heart) a modification of the method by Ohwada

(1986). The samples were collected by the help of 5mls syringe attached to needle (21 SWG) into plain capped bottles containing ethylene diamine tetraacetate (EDTA). The samples were immediately used for the estimation of the different variables.

Measurement of blood parameters

Blood samples were analyzed using automated cell counter (Coulter Electronics, Luton, Bedfordshire, UK) with standard calibration according to the manufacturer's instruction (Coulter Electronic, 1979) using normal human blood and with complete profile for red blood cell (RBC) count, total white blood cell (WBC) count, differential WBC count, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR)

Statistical Analysis

Data were presented as mean ± SD. Data were analysed using a one way analysis of variance (ANOVA) then followed with post hoc test (Least Square Deviation). P value of less than 0.05 was declared as significant statistically.

RESULTS

Effect of chloroquine and coartem on RBC, PCV and Hb on days 3 and 7 of administration in rats

On day 3 of the experiment, no significant differences were observed in the RBC count, Hb and PCV of the different groups, Figs. 1 to 3. The RBC count ($x10^6$ cells/µL), Hb (g/dL) and PCV (%) of control group on day 3 were 6.22±0.51, 12.82±0.77 and 42.32±2.51 respectively. But on day 7, the red blood cell count of coartem recipients (6.05±0.31) was significantly lower when compared with the control (7.20±0.21, $p<0.05$) and chloroquine (7.40±0.26, $p<0.01$) groups. Hb concentrations (g/dL) on day 7 was also significantly lower in the coartem (11.70±0.33) recipients when compared with the control (13.12±0.32, $p<0.05$) and chloroquine (13.90±0.42, $p<0.01$). Same results were obtained for the PCV (%) on day 7, it was significantly lower ($p<0.05$) in coartem (38.24±1.64) recipients compared with control (44.46±1.21) and chloroquine (49.68±3.21) groups.

Effect of chloroquine and coartem on platelet count and total WBC count on days 3 and 7 of administration in rats

As shown in Fig. 5, the mean platelet counts ($x10^3$cells/µL) in the control, coartem and chloroquine groups at day 3 were 558.20±110.94, 817.20 ±53.01 and 703.40±84.10 respectively, while their respective values at day 7 were 847.00±68.24, 674.40±53.41 and 851.00±56.43 respectively. The mean platelet counts were not statistically significant among the different groups both at day 3 and day 7 of administration.

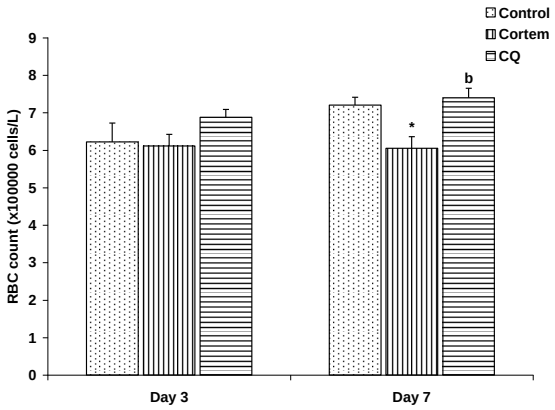

Fig. 1
Erythrocyte counts in control rats and those treated with Choloroquine and Coartem 3 and 5 days after treatment. Values are Mean SEM of five animals *p<0.05 (vs control); [b]P<0.05 (vs Coartem)

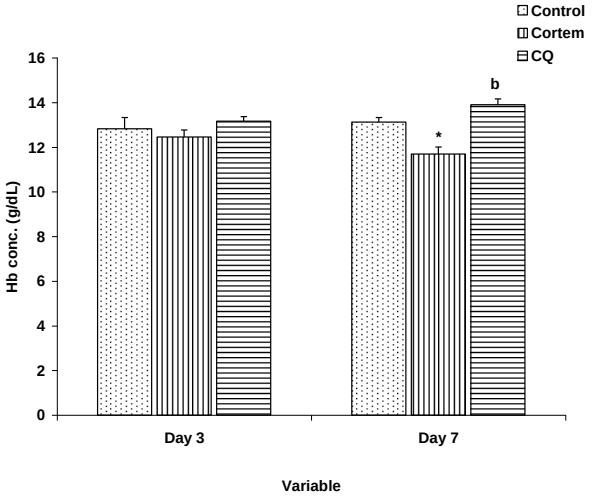

Fig. 2 Comparison of Hb concentration in the different experimental groups after 3 and 7 days of treatment. Values are mean ± SEM, n = 5. *p<0.05 vs control; b = p<0.01 vs Coartem

Fig. 2
Hemoglobin concentration in control rats and those treated with Choloroquine and Coartem 3 and 5 days after treatment. Values are Mean SEM of five animals *p<0.05 (vs control); [a]P<0.05 (vs Coartem)

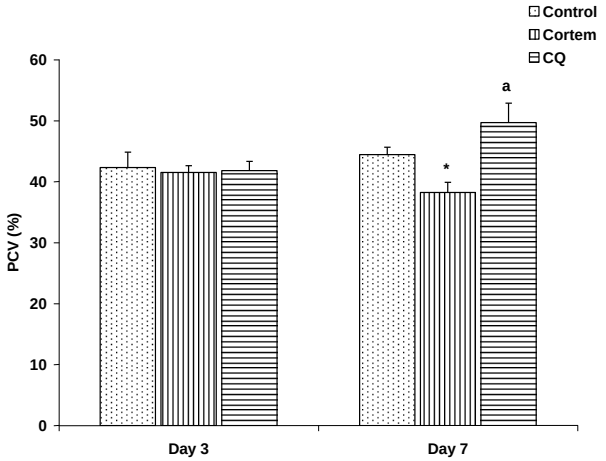

Fig. 3
Packed Cell Volume in control rats and those treated with Choloroquine and Coartem 3 and 5 days after treatment. Values are Mean SEM of five animals *p<0.05 (vs control); [a]P<0.05 (vs Coartem)

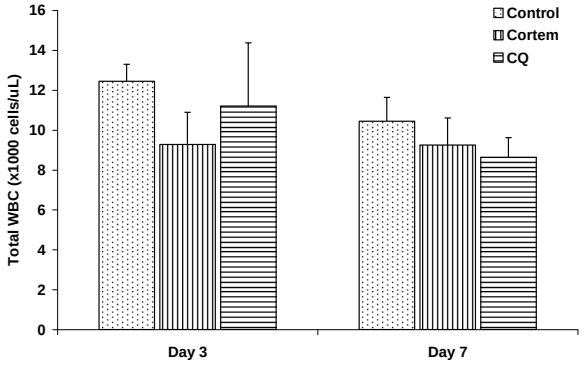

Fig. 4
Leucocyte counts in control rats and those treated with Choloroquine and Coartem 3 and 5 days after treatment. Values are Mean SEM of five animals *p<0.05 (vs control); [a]P<0.05 (vs Coartem)

Effect of Chloroquine and Coartem on RBC indices on days 3 and 7 of administration in rats

Results on the effects of chloroquine and coartem on RBC indices (MCV, MCH, MCHC, RDW-SD and RDW-CV) are summarized in table 1.

On day 3, the MCV (fL) for control was 68.64±2.12, values for coartem, and chloroquine groups were 68.18±1.73, and 60.80±0.98 respectively, showing significant decrease in chloroquine compared with control ($p<0.05$) and coartem ($p<0.01$) groups. Also, the MCH was significantly lower in chloroquine compared with control ($p<0.05$) and coartem ($p<0.01$) groups, while MCHC for control, coartem, and chloroquine were not significantly different.

On day 7, MCV (fL), MCH (pg) and MCHC (g/dL) for the control were 61.56±0.70, 18.26±0.29 and 29.72±0.59 respectively. No significant differences were observed in MCV, MCH and MCHC of the different groups on day 7.

The RDW-SD (fL) in the control, coartem and chloroquine at day 3 were 44.64±4.46, 42.08±2.28 and 34.32±1.66 respectively, showing significant decrease ($p<0.05$) in chloroquine recipients compare to coartem recipients. But at day 7, no significant differences were observed among the different groups.

The RDW-CV values in the control, coartem and chloroquine groups at day 3 were 15.44±1.50, 16.24±0.7 and 14.54±0.81 (fL) respectively, showing no significant differences. At day 7 their respective values were 17.98±1.38, 15.62±1.05 and 14.68±0.25 (fL), showing significant ($p<0.05$) decrease in chloroquine recipients compared with control..

Table 1
Effect of Chloroquine and Coartem on RBC and platelet indices on day 3 and 7 of administration in rats

	Day 3			Day 7		
	Control	**Coartem**	**CQ**	**Control**	**Coartem**	**CQ**
MCV (fL)	68.64±2.12	68.18±1.73	60.80±0.98*, b	61.56±0.70	63.34±0.90	59.50±2.52
MCH (pg)	20.80±0.60	20.36±0.14	19.14±0.31*, b	18.26±0.29	19.46±0.60	18.82±0.23
MCHC (g/dL)	30.32±0.53	29.96±0.71	31.52±0.38	29.72±0.59	30.68±0.57	30.46±0.39
RDW-SD (fL)	44.64±4.46	42.08±2.28	34.32±1.66[a]	39.42±2.34	37.36±2.00	34.84±0.88
RDW-CV (%)	15.44±1.51	16.24±0.71	14.54±0.81	17.98±1.38	15.62±1.05	14.68±0.25*
PDW (fL)	9.94±1.11	8.82±0.18	8.92±0.43	8.46±0.43	8.56±0.34	8.62±0.32
MPV (fL)	7.52±0.40	7.28±0.06	7.30±0.24	6.90±0.24	7.06±0.14	7.04±0.15
P-LCR (%)	11.44±2.51	9.10±0.39	9.32±1.44	6.90±0.99	7.84±0.67	7.54±1.08

Values are mean ± SEM, n = 5. *p<0.05 vs control; a = p<0.05, b = p<0.01 vs coartem

Table 2:
Effect of Chloroquine and Coartem on differential WBC count on day 3 and 7 of administration in rats.

	Day 3			Day 7		
	Control	Cortem	CQ	Control	Cortem	CQ
Lymphocytes (%)	83.40±5.85	84.86±4.61	81.60±4.24	76.00±8.06	77.80±4.82	74.20±3.60
Eosinophils (%)	1.00±0.45	2.20±0.97	2.00±0.45	2.00±0.55	1.80±0.58	1.60±0.51
Neutrophils (%)	15.40±5.41	11.00±3.52	16.20±3.98	21.20±7.27	19.60±4.58	23.60±3.04
Monocytes (%)	0.20±0.20	0.20±0.20	0.20±0.20	0.80±0.37	0.80±0.37	0.60±0.40

Values are mean ± SEM, n = 5.
No significant differences were observed between days 3 and 7

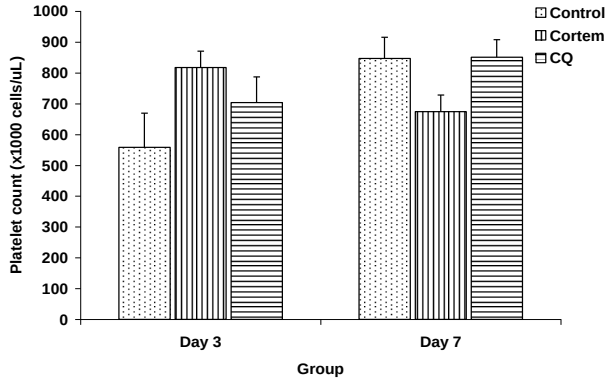

Fig. 4
Platelet counts in control rats and those treated with Choloroquine and Coartem 3 and 5 days after treatment. Values are Mean SEM of five animals *p<0.05 (vs control); [a]P<0.05 (vs Coartem)

Effect of chloroquine and coartem on platelet indices on days 3 and 7 of administration in rats

The effects of chloroquine and coartem platelet indices (MPV, P-LCR and PDW) are also summarized in table 1. The PDW in the control, coartem and chloroquine at day 3 were 9.94±1.11, 8.82±0.18 and 8.92±0.43 (fL) respectively, while respective values at day 7 were 8.46±0.43, 8.56±0.34 and 8.62±0.32 respectively. No significant differences were also observed among the different experimental groups at both days 3 and 7 of administration. No significant differences were also observed in MPV and P-LCR following administration of chloroquine and coartem

Effect of chloroquine and coartem on differential WBC counts on days 3 and 7 of administration in rats

Results on differential WBC count are shown in table 2. No significant differences were observed in the lymphocytes, neutrophils, eosinophils and monocytes counts following administration of chloroquine and coartem on days 3 and 7.

DISCUSSION

Chloroquine is one of the oldest known anti-malaria drugs but due to resistance of malaria parasites, especially *P. falciparum* to chloroquine, new therapeutic regimens have been compounded. Recently, WHO recommends the use of artemisinin-based combination therapies (ACTs) as first-line treatment of uncomplicated *Plasmodium falciparum* malaria, (WHO, 2010). ACTs therapy is currently regarded more effective relative to non-artemisinin regimens like chloroquine, and also yielding rapid symptomatic improvement and parasite clearance and a reduction in gametocyte carriage, which could help to reduce malaria transmission (Targett *et al*., 2001; Kokwaro *et al*., 2007; Premji, 2009).

These drugs when administered are carried in the blood stream where their actions are executed. Obviously, these drugs are without side effects, hence this study aimed to elucidate comparatively the effect of chloroquine and coartem on haematological parameters after 3 and 7 days of administration in rats.

Results obtained from this study reveal that these drugs do not have tremendous or serious adverse effects on blood parameter after 3 days of administration, the red blood cell count, Hb and PCV were not significantly altered following administration of the drugs after 3 days, but after 7 days of administration, the RBC count, Hb and PCV were significantly reduced in coartem recipients compared with control. Coartem appeared to have no deleterious effect on red blood cell, Hb and PCV when administered at the recommended dose for 3 days. But there is an indication that prolonged administration of coartem would possibly lead to anaemia. This is in consonance with reports from preclinical data suggesting that repeated exposure to coartem may affect blood cell counts and predispose to anaemia (Obianine *et al*, 2011).

However, chloroquine did not have any significant influence on the RBC, Hb and PCV both after

3 and 7 days of administration but it rather lead to a reduction in MCV and MCH, a possible indication that administration of chloroquine for 3 days could lead to microcytic anaemia.

The RDW-SD was also significantly reduced in chloroquine recipients relative to coartem administered group. RDW-SD is a numerical measure of the variability in size (anisocytosis) of circulating erythrocytes (Perkins, 2003). This parameter is routinely reported as part of the complete blood count but its use is generally restricted to narrowing the differential diagnosis of anaemia (McKenzie, 2003). There is also a strong correlation between RDW and risk of adverse outcome of heart failure (Felker *et al*., 2007). It is also elevated in thrombotic thrombocytopenic purpura, a disease of unknown origin, characterised by abnormally low levels of platelets in the blood, formation of blood clot in the arterioles and capillaries of many organ and neurological damage.

Results obtained from this study on platelet counts and platelet indices indicate that neither chloroquine nor coartem has any tremendous effect on platelets, no significant alterations in platelet count and platelet indices were observed following administration of these drugs for 3 and 7 days. This observation is contrary to earlier report by Ashley (2008), that coartem drug may cause low platelet count which may lead to bleeding tendencies in patients, the drugs may also cause symptoms that are related to low platelet count. Nevertheless, Low mean platelet volume measurements are relatively rare and may be associated with serious illnesses such as leukemia.

Platelets play an important role in the integrity of normal homeostasis and mean platelet volume (MPV) is the indicator for its function (Jakubowski *et al*., 1983), including aggregation, release of thromboxane A_2, platelet factor 4, beta-thromboglbulin (Martin and Bath, 1991; Sharp *et al*., 1995) and expression of glycogen 1b and glycogen IIb/IIIa receptors (Tschoepe *et al*., 1990; Giles *et al*., 1994).

MPV was not significantly altered in this study following administration of the two anti-malaria drugs. MPV a determinant of platelet function; is a newly emerging risk factor for atherothrombosis. Increase in MPV has been documented in patients with metabolic syndrome, stroke and diabetes mellitus (DM) (O'Malley and Langhorne, 1995; Tavil *et al*., 2007). Many studies have shown that increased MPV is one of the risk factors for myocardial infarction, cerebral ischaemia and transient ischaemic attacks (Khandekar *et al*., 2006; Kiliçli-Camur *et al*., 2005; Nadar *et al*., 2004; McCabe *et al*., 2004) and chronic vascular disease (Endler *et al*., 2002).

The total white blood cell (leucocyte) and differential counts were not significantly altered following administration of the chloroquine and coartem throughout the duration of the experiment. Our finding is at variance with earlier report by Adeleye *et al*., (2012) that coartem increases total WBC and lymphocyte count but decreases neutrophils count, they attributed these changes to immunological response induced by the drug (Guyton and Hall, 2006).

In conclusion, administration of chloroquine and coartem at their recommended doses for 3 days does not adversely alter the levels of RBC, total & differential WBC, Hb, PCV, platelet count and platelet function indices, except for MCV and MCH reductions in chloroquine recipients. However, after 7 days of administration, coartem causes reduction in RBC count, Hb and PCV. Hence, this drugs should be used as prescribed by a certified Physician.

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