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Evaluation of Short-Term Haematological and Nephro-Toxic Effects of Chloroquine, and Artemether-Lumefantrine in Albino Rats

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Abstract
The present study was designed to evaluate of short-term haematological and nephrotoxic effects of chloroquine, and artemether-lumefantrine in albino rats. Sixty adult male albino rats, 12–13 weeks of age, weighing 156 – 179 g were procured and used for this study. The rats were assigned into five groups of twelve rats per group replicated 3 times (4 rats per replicate). The groups were: 1. control group (CONTL GRP), 2. high dose artemether Lumefantrine (HD ARTEM LUMF. 4/24 mg/ml), 3. low dose artemether-lumefantrine (LD ARTEM LUMF. 2/12 mg/ml), 4. high dose chloroquine (HD CHLQN. 20 mg/ml) and 5. low dose chloroquine (LD CHLQN. 10 mg/ml)). Rats in the Control group were administered an equivalent volume of placebo (distilled water) according to body weight. Treatment was done daily and lasted for 3 days. The administration was orally using plastic syringes attached to a metal oropharyngeal cannula. Both doses of chloroquine (20 mg/ml and 10 mg/ml) did not have a significant effect on various blood parameters (WBC, Neu, Lym, Mon, Eos, Bas, RBC, HGB, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV and PDW) in rats, except for HCT and PCT, where both doses caused a significant increase. Similarly, high and low doses of artemether-lumefantrine (4/24 mg/ml and 2/12 mg/ml) and chloroquine did not significantly affect urea and creatinine levels in rats compared to the control. The administration of chloroquine and artemether-lumefantrine did not have a significant impact on blood parameters and renal function in rats, except for a notable increase in HCT and PCT.
Keywords: Artemether-Lumefantrine, Chloroquine, Haematology, Nephrotoxicity, Effects, Rats
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Introduction

The dearth in the literature on specifically the short-term haematological and nephrotoxic effects of anti-malaria drugs especially chloroquine and artemether-lumefantrine prompted the need to embark on the present study. This study was conducted to investigate the potential adverse effects of these antimalarial drugs on the blood and kidneys of laboratory rats. The study aims to provide a comprehensive understanding of the hematological changes and nephrotoxicity induced by chloroquine and artemether-lumefantrine, which are commonly used medications for the treatment of malaria.

Chloroquine has been a widely used antimalarial drug for several decades (Hsiang et al., 2012). It is known to exhibit antiparasitic activity by interfering with the heme detoxification pathway in Plasmodium species (Thomé et al., 2013). Despite its efficacy against malaria, there have been reports of detrimental effects on the host (Uzochukwu et al., 2010, Muheet et al., 2014).

Artemether-lumefantrine is another combination therapy commonly used for the treatment of malaria (Ijeomah et al., 2016). This drug combination acts by inhibiting the replication of the malaria parasite within red blood cells (Loon et al., 2022). Although artemether-lumefantrine is generally considered safe and well-tolerated, there have been concerns regarding its potential adverse effects on the hematological system and kidneys (Baiden et al., 2015).

Given the widespread use of chloroquine and artemether-lumefantrine in malaria-endemic regions, it is essential to evaluate their short-term effects on blood parameters and kidney function. The findings of this study may contribute to a better understanding of the potential risks associated with these antimalarial drugs and help guide clinical decision-making regarding their use in the treatment of malaria.

Materials and methods

Equipment used

Rat cages equipped with drinking and feeding facilities, artemether-lumefantrine and chloroquine tablets, digital weighing balance (Metler H_3O, Switzerland), and plastic syringes attached to the metal oropharyngeal cannula.

Procurement of drugs
Lokmal QS-Combl Artemether 80 mg\Lumefantrine 480 mg and chloroquine 250 mg manufactured by Emzor Pharmaceutical Industries Limited Flower Gate Mixed Development Scheme, KM 1, Sagamu Benin Expressway, Makun-Sagamu, Ogun State, Nigeria was bought from reputable pharmaceutical store.

Procurement and management of experimental animals
Sixty adult male albino rats, 12–13 weeks of age, weighing 156 – 179 g were procured from the Genetics and Experimental Animal Breeding Laboratory of Zoology and Environmental Biology Department, University of Nigeria, Nsukka were used for this investigation. The rats had no history of drug consumption (i.e., they have not been used for any investigation). They were kept in stainless wire rat cages equipped with drinkers and fecal collecting trays, in a clean and fly-proof experimental animal house. The rats were fed with commercial grower's chick mash made by Vital Feeds, Nigeria Limited, and clean drinking water. They were allowed to acclimatize for fourteen days before the start of the experiment. The rats were allowed unhindered access to food and water. The fecal droppings in the tray were removed daily.

Experimental design

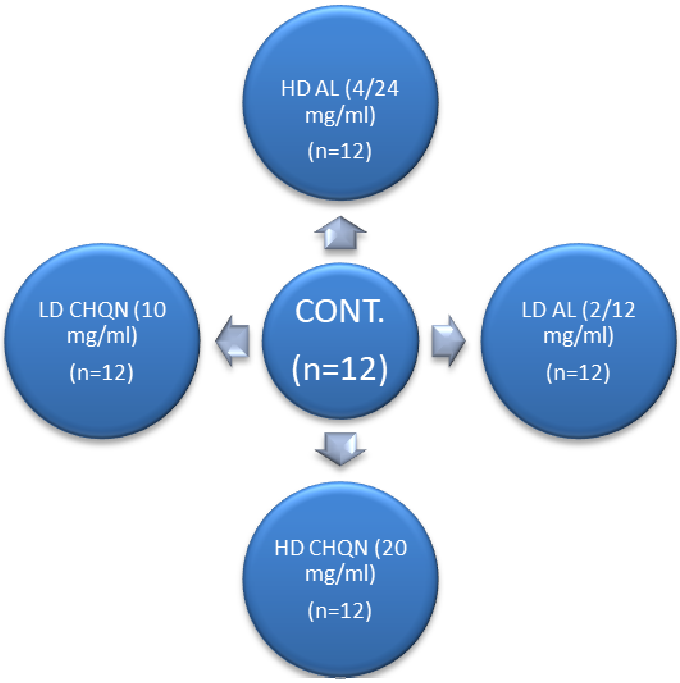

Figure 1. Experimental design (CONT. = Control; HD AL = HIGH DOSE Artemether Lumefantrine; LD AL = LOW DOSE Artemether Lumefantrine; HD CHQN = HIGH DOSE Chloroquine; LD CHQN = LOW DOSE Chloroquine)

The study adopted the experimental design. It was a randomized complete block design with each group replicated 3 times (4 rats per replicate). The rats were assigned into five groups of twelve rats per group (1. CONTROL GROUP (CONTL GRP), 2. HIGH DOSE Artemether Lumefantrine (HD ARTEM LUMF. 4/24 mg/ml), 3. LOW DOSE Artemether Lumefantrine (LD ARTEM LUMF. 2/12 mg/ml), 4. HIGH DOSE Chloroquine (HD CHLQN. 20 mg/ml) and 5. LOW DOSE Chloroquine (LD CHLQN. 10 mg/ml)). Rats in the Control group were administered an equivalent volume of placebo (distilled water) according to body weight. Treatment was done daily and lasted for 3 days. The administration was orally using plastic syringes attached to the metal oropharyngeal cannula.

Collection of blood sample
About 5 ml of the blood samples was collected from each of the anaesthetized rats using the method described by Hoff, 2000.

Measurement of packed cell volume (PCV):
The hematocrit method, as described by Coles in 1986, was employed to determine the packed cell volume (PCV) in this study. The results were presented as percentages.

Assessment of white blood cell (WBC) and red blood cell (RBC) counts:
For the enumeration of white blood cells (WBC) and red blood cells (RBC), an improved Neubauer chamber, as outlined by Coles in 1986, was utilized. The cell counts were expressed as x 106 counts/µL of blood.

Determination of hemoglobin (Hb) concentration:
To determine the hemoglobin (Hb) concentration, the cyanomethemoglobin method described by Coles in 1986 was employed. The recorded values were in units of g/dL.

Differential leukocyte count:
The Lieshmann technique, as detailed by Coles in 1986, was employed for the differential leukocyte count. Each leukocyte type was assessed as a percentage of the total count and then converted to an absolute value per microliter of blood.

Serum creatinine
Serum Creatinine was determined using the alkaline picrate method (Jaffe's Method) as described by Toora & Rajagopal, 2002.

Serum urea
Serum urea was determined using the diacetyl monoxime method as described by Rahmatullah & Boyde, 1980.

Statistical Analysis
Data analysis was carried out with a statistical package for social sciences SPSS, IBM Statistics UK version 16.0 one–way analysis of variance (ANOVA). The means were separated using Duncan's new multiple range test while differences in the means were considered significant at probability values less than 5 % ($p < 0.05$). The results were presented as mean ± SEM.

RESULTS

Effects of chloroquine on haematological profile of normal albino rats
Both 20 mg/ml and 10 mg/ml doses of chloroquine administered daily to normal albino rats based on body weight showed a non-significant effect ($p > 0.05$) in WBC, Neu, Lym, Mon, Eos, Bas, RBC, HGB, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, and PDW except in HCT and PCT were both high (20 mg/ml) and low (10 mg/ml) doses of chloroquine administered caused a significant increase ($p < 0.05$) compared with the control after 3 days of treatment (Table 1).

Effects of artemether lumefantrine and chloroquine on urea and creatinine
A similar trend was observed on the effects of artemether lumefantrine and chloroquine on nephrotoxicity after a 3-day treatment in normal albino tars. Both high (4/24 mg/ml) and low (2/12 mg/ml) doses of artemether-lumefantrine caused no significant effect ($p > 0.05$) on urea and creatinine as compared with the control.
Similarly, the two doses (20 mg/ml & 10 mg/ml) of chloroquine administered showed no significant effect ($p > 0.05$) on urea and creatinine as compared with the control (Table 2).

Table 1 Effects of chloroquine on haematological parameters in rats

Groups	WBC	Neu	Lym	Mon	Eos	Bas	RBC	HGB	HCT	MCV	MCH	MCHC	RDW-CV	RDW-SD	PLT	MPV	PDW	PCT
Control	11.23 ± 1.93 [a]	3.86 ± 1.15 [a]	7.30 ± 0.95 [a]	0.06 ± 0.02 [a]	0.01 ± 0.00 [a]	0.00 ± 0.00 [a]	7.42 ± 0.36 [a]	13.15 ± 0.67 [a]	38.70 ± 1.91 [a]	52.25 ± 1.67 [a]	17.73 ± 0.55 [a]	33.98 ± 0.09 [a]	0.15 ± 0.03 [a]	29.20 ± 8.91 [a]	603.00 ± 67.99 [a]	7.60 ± 0.15 [a]	15.48 ± 0.09 [a]	3.82 ± 0.44 [a]
High Dose CHQN	11.73 ± 0.77 [a]	2.85 ± 0.58 [a]	7.30 ± 2.23 [a]	0.07 ± 0.04 [a]	0.01 ± 0.01 [a]	0.00 ± 0.00 [a]	8.02 ± 0.54 [a]	15.00 ± 0.70 [a]	44.53 ± 2.03 [b]	55.90 ± 2.07 [a]	18.78 ± 0.55 [a]	33.63 ± 0.35 [a]	0.20 ± 0.01 [a]	46.03 ± 2.96 [a]	651.75 ± 32.10 [a]	7.68 ± 0.17 [a]	15.58 ± 0.06 [a]	4.98 ± 0.14 [b]
Low Dose CHQN	9.60 ± 1.40 [a]	2.37 ± 0.30 [a]	7.22 ± 1.26 [a]	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]	0.00 ± 0.00 [a]	7.97 ± 0.39 [a]	14.50 ± 0.39 [a]	42.63 ± 0.68 [a,b]	53.73 ± 1.78 [a]	18.28 ± 0.49 [a]	34.03 ± 0.38 [a]	0.20 ± 0.00 [a]	43.93 ± 1.27 [a]	644.75 ± 33.63 [a]	7.83 ± 0.08 [a]	15.63 ± 0.03 [a]	5.04 ± 0.25 [b]

Table 2 Effects of artemether lumefantrine and chloroquine on urea and creatinine

Groups	UREA (mmol/L)	CREATININE (mmol/L)
Control	6.74 ± 0.43 [a]	57.60 ± 2.72 [a]
High Dose A/L	7.13 ± 0.17 [a]	60.17 ± 1.76 [a]
Low Dose A/L	7.61 ± 0.76 [a]	64.54 ± 4.13 [a]
High Dose CHLQN	6.87 ± 0.54 [a]	64.54 ± 2.88 [a]
Low Dose CHLQN	7.13 ± 0.48 [a]	61.17 ± 3.54 [a]

A/L - artemether lumefantrine; CHLQN – chloroquine

Discussion

Blood parameters can also be utilized to elucidate the blood-related functions of chemical compounds (Yakubu et al., 2007). Our study revealed that the administration of both doses of chloroquine (20 mg/ml and 10 mg/ml) did not have a significant effect ($p > 0.05$) on WBC, Neu, Lym, Mon, Eos, Bas, RBC, HGB, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, and PDW, except for HCT and PCT, where a significant increase ($p < 0.05$) was observed compared to the control group. These results indicate that the tested drugs did not cause any changes in the hematological parameters of the laboratory animals. Furthermore, there were no negative effects on the blood profile of the treated rats. However, the significant increase ($p<0.05$) in PCT could indicate the presence of an infection, while the significant increase ($p<0.05$) in HCT could suggest dehydration.

These findings are consistent with the results of Ofem et al. (2013), but they differ from the findings of Olomu et al. (2018). The disparity between our findings and those of Olomu et al. (2018) could be attributed to differences in the duration of treatment.

Creatinine and urea are sensitive biochemical markers used to assess renal function (Abolaji et al., 2013). Urea is produced from protein and amino acid catabolism, and its synthesis occurs exclusively through hepatic enzymes in the urea cycle. It is primarily cleared from the circulation by the kidneys. Therefore, kidney dysfunction leads to the accumulation of urea in the blood (Lamb & Price, 2008). Creatinine is formed from the spontaneous cyclization of creatine and creatine phosphate at a steady rate and is rapidly eliminated from the blood by the kidneys (Harvey & Ferrier, 2011). Measurement of serum concentrations of creatinine, urea, and uric acid is commonly used to evaluate kidney function and its conditions (Lamb & Price, 2008). In our study, it was observed that both high (4/24 mg/ml) and low (2/12 mg/ml) doses of artemether-lumefantrine, as well as both doses (20 mg/ml & 10 mg/ml) of chloroquine, did not have a significant effect ($p > 0.05$) on urea and creatinine levels compared to the control group. This indicates that the administration of the anti-malaria drugs for three days did not alter the levels of urea and creatinine. Consequently, the excretory function of the kidneys was not adversely affected by the three-day administration of artemether-lumefantrine and chloroquine. These results suggest that the kidneys of the tested animals were functioning normally and that there was no impairment of renal function. Our findings align with the results reported by Abolaji et al. (2013), but they differ from those of Omoboyowa et al. (2018).

Conclusion

The administration of chloroquine and artemether-lumefantrine did not have a significant impact on blood parameters and renal function in rats, except for a notable increase in HCT and PCT.

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Conflict of interest: The authors declare that they have no conflict of interest.

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