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Evaluation of Short-Term Effects of Anti-Malaria Drugs on Haematology and Serum Electrolytes in Rats

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Abstract
The study investigated the short-term effects of artemether-lumefantrine and chloroquine on haematology and serum electrolytes profile 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 4/24 mg/ml and 2/12 mg/ml doses of artemether-lumefantrine showed a non-significant effect ($p > 0.05$) in WBC, Neu, Lym, Mon, Eos, Bas, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, and PDW except in PCT where low (2/12 mg/ml) dose caused a significant increase ($p < 0.05$) compared with the control. Artemether-lumefantrine and chloroquine had similar effects on K, Na, Cl, iCa, TiCa, TCa, pH, TCO_2, and AG. Both high (4/24 mg/ml) and low (2/12 mg/ml) doses of artemether-lumefantrine showed no significant effect ($p > 0.05$) on K, Na, Cl, iCa, TiCa, TCa, pH, TCO_2, and AG but Cl showed a significant increase ($p<0.05$) compared with the control. Chloroquine doses (20 mg/ml & 10 mg/ml) had similar effects except on Cl where the high dose (20 mg/ml) caused a significant increase ($p<0.05$) as compared with the control. However, the significant increase in Cl could depict dehydration.
Keywords: Artemether-Lumefantrine, Chloroquine, Effects, Haematology, Electrolytes, Rats
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Introduction

Malaria continues to be a significant global health issue, particularly in tropical and subtropical regions, affecting millions of people each year. The disease is caused by Plasmodium parasites transmitted through the bite of infected mosquitoes. Antimalarial drugs play a crucial role in the treatment and prevention of malaria, targeting the parasites and helping to control the spread of the disease (World Health Organization [WHO], 2021.

While antimalarial drugs have proven to be effective in combating malaria, they can also have various side effects on the human body. These side effects may range from mild symptoms such as nausea, headaches, and dizziness, to more severe complications affecting vital organs and body systems (Mulenga-Cilundika et al., 2020, Nnamonu et al., 2020).

To ensure the safe and effective use of antimalarial drugs, it is essential to thoroughly evaluate their short-term effects on key physiological parameters. Haematology and serum electrolytes are vital indicators of the overall health status and functioning of an organism. Changes in these parameters can provide valuable insights into the potential adverse effects of antimalarial drugs on the blood and electrolyte balance in the body (Bishop et al., 2010, Anago et al., 2016).

Although several studies have investigated the long-term effects of antimalarial drugs, there is a relative scarcity of research focusing specifically on the short-term effects of these drugs on haematological parameters and serum electrolytes. Understanding the immediate impact of antimalarial drugs on these biomarkers in a controlled animal model can help in identifying any potential risks or imbalances that may arise during the early stages of drug administration. The aim of this study, therefore, is to evaluate the short-term effects of commonly used antimalarial drugs on haematology and serum electrolytes in rats. By conducting this research, we hope to gain a better understanding of the immediate hematological and electrolyte changes induced by antimalarial drugs, which can aid in predicting and managing potential adverse effects in humans.

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

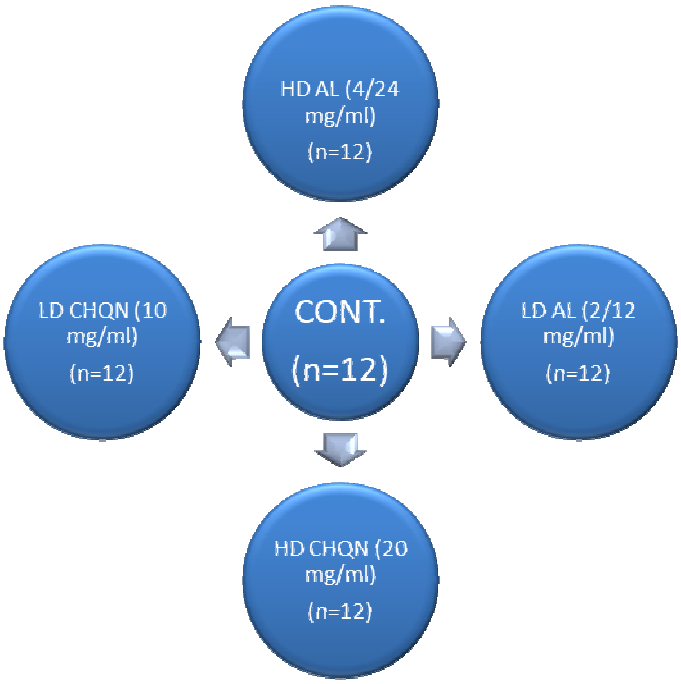

Figure 1. Experimental design (CONT. = Control; HD AL = HIGH DOSE Artemether Lumefantrine; LD AL = LOW DOSE Artemether Lumefantrine; HD CHQN = HIGH DOSE Chloroquine; LD CHQN = LOW DOSE Chloroquine)

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.

Blood Sample Collection

To obtain blood samples, orbital techniques were employed for haematology analysis. The retrobulbar plexus of

the median canthus of the animals' eyes was used to collect the blood samples using capillary tubes. These samples were then transferred into plastic bijoux bottles containing EDTA for haematology analysis. The collected blood samples underwent analysis for various parameters including Packed Cell Volume (PCV), Erythrocyte count (RBC), Haemoglobin concentration (HB), Total White Blood Cell count (WBC), and Differential White Blood Cell count.

Determination of Packed Cell Volume (PCV)
The determination of PCV utilized the micro haematocrit method described by Thrall & Weiser (2002) and Coles (1986). The capillary tubes were filled with blood samples up to three-quarters full through capillary action. One end of the tubes was sealed with plasticine, and they were then placed in a micro haematocrit centrifuge. The centrifugation was carried out at 11,000 revolutions per minute for 5 minutes using the micro haematocrit centrifuge. The PCV was measured as a percentage using a micro haematocrit reader.

Red Blood Cell Count
The count of red blood cells was performed using the haemocytometer method, as described by Thrall & Weiser (2002) and Coles. A micropipette was used to draw 0.02 ml of the blood sample, which was then mixed with 4 ml of erythrocyte-diluting fluid in a test tube. A drop of the mixture was placed in the Neubauer chamber to charge it. The erythrocytes were counted under a microscope using an x40 objective and a tally counter. The count was conducted in five squares, including four at the edges and one at the center. The obtained count was multiplied by a factor of 10,000 to determine the absolute count of erythrocytes per microliter of blood.

Determination of Haemoglobin Concentration
The haemoglobin concentration was determined using the cyanomethaemoglobin method described by Higgins et al. (2008). For this analysis, 5 ml of Drabkin's haemoglobin reagent was added to a clean test tube. Subsequently, 0.02 ml of the blood sample was added to the reagent and thoroughly mixed. The mixture was allowed to react for 20 minutes, and the absorbance was measured at a wavelength of 540 nm using a spectrophotometer, against a reagent blank. Standards were prepared in the same manner and also read at 540 nm. The haemoglobin concentration of the blood sample was calculated by multiplying the sample's absorbance by a calibration factor derived from the absorbance and concentration of the mean of the standards.

Total White Blood Cell Count
The count of white blood cells was conducted using the haemocytometer method described by Thrall & Weiser (2002) and Coles. Similarly, 0.02 ml of the blood sample was drawn using a micropipette and mixed with 0.4 ml of white blood cell diluting fluid in a test tube. The Neubauer chamber was then charged with a drop of the mixture and examined using a microscope with an x10 objective. White Blood Cells (Leukocytes) were counted in the four squares at the edges of the chamber using a tally counter. The obtained count of leukocytes was multiplied by a factor of 50 to determine the total number of leukocytes per microliter of blood.

Differential White Blood Cell Count
The differential count of white blood cells was performed using the Leishmann technique as described by Thrall & Weiser (2002). A drop of blood was gently shaken and carefully smeared on a clean grease-free slide using a cover slip, creating a thin smear. The smear was air-dried and then stained using the Leishmann stain and examined under a light microscope using an immersion objective. By employing the longitudinal counting method, 200 cells were counted, and each cell type was identified and recorded using the differential cell counter. The results for each type of white blood cell were expressed as a percentage of the total count and converted to the absolute value per microliter of blood.

Determination of serum electrolytes
Serum electrolytes (Potassium (K), Sodium (Na), Chloride (Cl), Ionized Calcium (iCa), Total Ionized Calcium TiCa, pH, TCO_2, and Silver (Ag)) were determined using the method of Sood, 2006.

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 artemether-lumefantrine on haematological profile of normal albino rats

The effects of artemether-lumefantrine on the haematological profile of normal albino rats are presented in Table 1. Both 4/24 mg/ml and 2/12 mg/ml doses of artemether-lumefantrine 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, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, and PDW except in PCT where low (2/12 mg/ml) dose of artemether-lumefantrine administered caused a significant increase ($p < 0.05$) compared with the control after 3 days of treatment.

Effects of artemether-lumefantrine and chloroquine on serum electrolytes

The effects of artemether-lumefantrine and chloroquine on serum electrolytes are shown in Table 2. A similar trend was observed on the effects of artemether-lumefantrine and chloroquine on serum electrolytes profile such as K, Na, Cl, iCa, TiCa, TCa, pH, TCO_2, and AG after a 3-day treatment in normal albino rats. Both high (4/24 mg/ml) and low (2/12 mg/ml) doses of artemether-lumefantrine showed no significant effect ($p > 0.05$) on all parameters tested except on Cl where both doses caused a significant increase ($p<0.05$) 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 all parameters investigated except on Cl where the high dose (20 mg/ml) caused a significant increase ($p<0.05$) as compared with the control.

Table 1 Effects of artemether-lumefantrine 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
CON	11.23 ± 1.93 [a]	3.86 ± 1.15 [a]	7.30 ± 0.95 [a]	0.06 ± 0.02 [b]	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 A/L	12.51 ± 1.06 [a]	2.57 ± 0.39 [a]	9.90 ± 1.05 [a]	0.01 ± 0.00 [a]	0.02 ± 0.01 [a]	0.00 ± 0.00 [a]	7.81 ± 0.23 [a]	13.95 ± 0.18 [a]	41.95 ± 0.31 [a]	53.88 ± 2.03 [a]	17.90 ± 0.43 [a]	33.28 ± 0.52 [a]	0.21 ± 0.01 [a]	45.25 ± 2.38 [a]	622.50 ± 29.74 [a]	7.83 ± 0.10 [a]	15.53 ± 0.09 [a]	4.90 ± 0.30 [a,b]
Low Dose A/L	14.75 ± 1.20 [a]	3.64 ± 0.52 [a]	11.08 ± 1.59 [a]	0.02 ± 0.01 [a]	0.02 ± 0.01 [a]	0.00 ± 0.00 [a]	7.52 ± 0.24 [a]	13.85 ± 0.06 [a]	41.85 ± 0.10 [a]	55.80 ± 1.78 [a]	18.50 ± 0.68 [a]	33.15 ± 0.23 [a]	0.21 ± 0.02 [a]	46.68 ± 2.88 [a]	694.25 ± 50.28 [a]	7.58 ± 0.13 [a]	15.65 ± 0.05 [a]	5.35 ± 0.30 [b]

Effects of artemether lumefantrine and chloroquine on electrolytes

Table 2 Effects of artemether lumefantrine and chloroquine on electrolytes

Groups	K (mmol/L)	Na (mmol/L)	Cl (mmol/L)	iCa (mmol/L)	TiCa (mmol/L)	Tca (mmol/L)	pH	TCO2 (mmol/L)	AG (mmol/L)
Control	5.97 ± 0.22 [a]	146.40 ± 1.98 [a]	97.38 ± 0.66 [a]	0.87 ± 0.04 [a]	1.21 ± 0.04 [a,b]	2.43 ± 0.08 [a,b]	8.33 ± 0.05 [a]	16.33 ± 2.06 [a]	32.68 ± 2.32 [a]
High Dose A/L	5.72 ± 0.24 [a]	143.00 ± 1.42 [a]	101.40 ± 0.88 [b]	0.95 ± 0.02 [a]	1.25 ± 0.02 [a,b]	2.51 ± 0.03 [a,b]	8.26 ± 0.06 [a]	15.40 ± 0.92 [a]	26.20 ± 2.63 [a]
Low Dose A/L	6.21 ± 0.41 [a]	145.68 ± 0.84 [a]	101.10 ± 1.55 [b]	0.90 ± 0.08 [a]	1.23 ± 0.03 [a,b]	2.45 ± 0.06 [a,b]	8.32 ± 0.10 [a]	12.00 ± 1.86 [a]	32.58 ± 1.79 [a]
High Dose CHQN	5.98 ± 0.20 [a]	144.93 ± 1.78 [a]	102.93 ± 1.01 [b]	0.86 ± 0.06 [a]	1.20 ± 0.05 [a]	2.40 ± 0.09 [a]	8.33 ± 0.04 [a]	10.60 ± 1.48 [a]	31.40 ± 1.92 [a]
Low Dose CHQN	6.18 ± 0.21 [a]	144.30 ± 1.53 [a]	100.25 ± 1.03 [a,b]	0.98 ± 0.07 [a]	1.32 ± 0.03 [b]	2.63 ± 0.06 [b]	8.32 ± 0.11 [a]	14.28 ± 2.30 [a]	29.80 ± 2.72 [a]

A/L - artemether lumefantrine; CHLQN - chloroquine

Discussion

It has been documented that blood parameters are good indicators of the physiological and nutritional status of animals; changes in blood parameters have been useful in elucidating the impact of nutritional factors and or additives supplied in the diets of living organisms and are also be used to explain blood relating functions of chemical compounds (Yakubu et al., 2007; Majid et al., 2010). This study provides valuable insights into the potential impact of anti-malaria medications on blood composition and electrolyte balance, contributing to the understanding of their safety and potential side effects in preclinical models and providing valuable insights for the development and optimization of malaria treatment strategies in humans.

The present study evaluated the effects of a 3-day artemether-lumefantrine treatment on the haematological profile of albino rats. The findings revealed that both doses of artemether-lumefantrine administered daily (4/24 mg/ml and 2/12 mg/ml) 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, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, and PDW except in PCT where low (2/12 mg/ml) dose of artemether-lumefantrine administered caused a significant increase ($p < 0.05$) compared with the control after 3 days of treatment. The above results imply that the testing drugs caused no haematological disorder nor any negative system assault. This simply shows that the 3-day treatment with artemether-lumefantrine did not cause any deleterious effect on the blood profile of the treated rats. However, the significant increase ($p<0.05$) in PCT could depict infection. Specifically, the result on WBC whole count and differential count implies that the tested anti-malaria drug did not affect the overall status of the immune system of the treated rats at the end of three days of treatment. The findings of Adeleye et al. (2012) and Osonuga et al. (2012) who investigated the effects of aoartem and artesunate on some haematological parameters such as RBC, WBC, differential WBC; Hb, and PCV in albino rats is in disagreement with the findings of the present study.

This study also investigated the effects of artemether-lumefantrine and chloroquine on serum electrolytes profile in albino rats. Our findings revealed a similar trend on the effects of artemether-lumefantrine and chloroquine on serum electrolytes profile such as K, Na, Cl, iCa, TiCa, TCa, pH, TCO_2, and AG after a 3-day treatment in normal albino rats. Both high (4/24 mg/ml) and low (2/12 mg/ml) doses of artemether-lumefantrine showed no significant effect ($p > 0.05$) on all parameters tested except on Cl where both doses caused a significant increase ($p<0.05$) 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 all parameters investigated except on Cl where the high dose (20 mg/ml) caused a significant increase ($p<0.05$) as compared with the control. These findings are at variance with Abolaji et al., 2013.

In an attempt to explain the above results, we tend to report that the normal level of K, Na, Cl, iCa, TiCa, TCa, pH, TCO_2, and AG observed points the anti-malaria drugs administered did not affect the normal serum levels of the electrolytes. It also depicts that the testing animals are healthy. However, the significant increase in Cl could depict dehydration.

Conclusion

The study found that both doses of artemether-lumefantrine (4/24 mg/ml and 2/12 mg/ml) did not have a significant effect on various blood parameters, except for a significant increase in PCT at the low dose. Artemether-lumefantrine and chloroquine doses administered had similar effects on several electrolyte levels, but the high dose of chloroquine caused a significant increase in Cl, which could indicate dehydration.

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

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