RESEARCH ARTICLE

Efficacy and safety properties of Lumefantrine-Trimethoprim-Copper complex in mice

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Abstract: Lumefantrine and trimethoprim are antimalarial and antibiotic drugs respectively. Even though, therapeutic agents have shown enhanced efficacy upon coordination to metal ion, antimalarial activity of lumefantrine-trimethoprim-copper complex drug has not been reported. This study evaluated the anti-malarial potency and safety of synthesized lumefantrine-trimethoprim-copper (LTC) complex in mice. A total of 35 albino mice were randomly divided into seven groups. Mice in groups 1 and 7 were not infected with *Plasmodium berghei*. Infected mice in groups 3, 4, 5, and 6 were treated with chloroquine, LTC, trimethoprim and lumefantrine respectively. All the animals were sacrificed 24 hours after completion of their doses. Percentage parasitaemia, chemo suppression attained in the group of mice infected, but treated with LTC (96.84%) or chloroquine (97.80%) was higher than those treated with lumefantrine (75.79%) or trimethoprim (76.84%) at day 8 post-inoculation, when compared with control. There was significant reduction in the activities of ALP, ALT and AST in the liver of treated mice when compared with control. Increased chromosomal aberration was observed in all treated groups when compared with control.The observed modifications in the biochemical indices and the presence of chromosomal aberrations in the organs studied, suggested a selective and functional toxicity of the drug.

Keywords: Lumefantrine-trimethoprim-copper, LTC, antimalarial, efficacy, safety.

INTRODUCTION

World Health Organization (WHO) reported that there were about 212 million incidences of malaria in 2015 and approximately 429 000 deaths in the sub-Saharan Africa due to malaria which is about 90% of all malaria deaths globally (WHO, 2015). Nigeria has been reported to have one-fourth of all malaria cases in Africa. Parasitic unicellular protozoans belonging to the genus *Plasmodium* cause malaria. This disease results when there is a bite from an infected female *Anopheles* mosquito introducing the parasite into the human circulatory system (WHO, 2015). During the past decade, the scale-up of malaria control interventions has resulted in considerable reductions in morbidity and mortality associated with malaria in parts of Africa (Steketee, 2010). Despite these efforts, malaria continues to pose a major public health threat in many African countries. The most effective strategy for the treatment of *P. falciparum* infection is the use of artemisinins in combination with other antimalarials (known as artemisinin-combination therapy), which reduces the ability of the parasite to develop resistance to any single drug component (Kokwaro, 2009). These additional antimalarials include amodiaquine, lumefantrine, mefloquine or sulfadoxine/pyrimethamine.

Lumefantrine is an antimalarial agent, usually used in combination with artemether for improved efficacy (Omari *et al.*, 2004). It is active against multi-drug resistant strains of *Plasmodia falciparum* and thereby effective for the treatment of various types of malaria. The mechanism of action of this combination therapy comprises activity against the erythrocytic stages of *Plasmodium* spp. Lumefantrine is an aryl- amino alcohol (2-(dibutylamino)-1-[(9Z)-2,7-dichloro-9-[(4-chlorophenyl)methylidene]fluoren-4-yl]ethanol) that averts detoxification of haem, such that the toxic haem and free radicals induce parasite death. Trimethoprim is an antibiotic used to treat various bacterial infections (Brogden *et al.,* 1982). It works by stopping the growth of bacteria. Trimethoprim binds to dihydrofolate reductase and inhibits the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF) (Brogden *et al.,* 1982). Consequently, reduction in the level of THF, an important precursor in thymidine synthesis pathway, will result in bacterial DNA synthesis inhibition. Trimethoprim and sulfamethoxazole are commonly used in combination due to possible synergistic effects, and reduced development of resistance (Brogden *et al.,* 1982). Not many works have been reported on the antimalarial activity of trimethoprim as a pure compound, and as a complex drug. Research has shown significant progress in the utilization of transition metal complexes as drugs to treat several diseases like carcinomas, lymphomas, diabetes and neurological disorders (Rafique *et al.,* 2010). Previous works have revealed that efficacy of

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some therapeutic agents increased upon coordination to transition metals (Arise *et al.*, 2016; Obaleye *et al.,* 2009a; Obaleye *et al.*, 2009b). This property of transition metals has enhanced the development of metal-based drugs with promising pharmacological application and may offer unique therapeutic opportunities (Rafique *et al.*, 2010). Various studies have been carried out on complexation of common anti-malaria drugs with metals (Obaleye *et al.*, 2009a, Arise *et al.,* 2017). The ability of the metal to combine with ligand and then release them in some process make them ideal for use in biological systems. Compounds that possess activity against both chloroquine-sensitive and chloroquine-resistant plasmodium strains have been produced from incorporation of metallocene into each of quinine and chloroquine (Obaleye *et al.,* 2009a).

In our previous work, we investigated the antimalarial activity of solvent-less synthesized lumefantrine-copper complex using mice infected with *P. berghei* NK-65 and reported that lumefantrine-copper complex has higher antimalarial activity than pure lumefantrine and also compared favourably with chloroquine (Arise *et al*., 2016). This study investigated the in *vivo* efficacy and safety of lumefantrine-trimethoprim-copper (LTC) complex in continuation of efforts to find new and safer antimalarial drugs effective against chloroquine resistant strain of malaria parasite.

MATERIALS AND METHODS

Drugs used

Lumefantrine-trimethoprim-copper complex was synthesized in the Department of Chemistry, University of Ilorin, Nigeria. Chloroquine phosphate capsules used is a product of the Tuyil Pharmaceutical Company, Ilorin, Nigeria. The active ingredient of lumefantrine and trimethoprim used are products of the Tuyil Pharmaceutical Company, Ilorin, Nigeria.

Experimental animals

Thirty five (35) white male albino mice with a mean weight of 19.75 g were obtained from the Animal Holding Unit of the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria.

Malaria parasite

Plasmodium berghei was obtained from the Institute for Advanced Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Nigeria.

Assay kits and reagents used

Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were from Randox Laboratories, United Kingdom. Analytical grade assay kits and reagents wereused.

Reflux synthesis of Lumefantrine-trimethoprim-copper complex

The reflux production of lumefantrine-trimethoprim-copper complex was carried out by dissolving 2mM of lumefantrine (0.22g) and 2mM of trimethoprim (0.12g) separately in dichloromethane and warm methanol respectively. One (1) milimoles of $CuCl_2.6H_2O$ (0.02g) was dissolved in methanol and mixed with ligand to give green solution. The resulting solution was refluxed at 60^0C for 1 hour and then filtered. On refluxing, the colour was changed to light green. The filtrate was subjected to slow evaporation at room temperature for two weeks, after which precipitate was formed. The precipitate was then filtered and dried.

Inoculation of experimental mice

25 Swiss albino mice were infected by intraperitoneal injection of standard inoculums (0.2ml of 1×10^7 infected erythrocytes from a single donor mouse previously infected with *Plasmodium berghei* (25% parasitaemia). Establishment of infection was confirmed after three days.

Experimental design

A total of 35 albino mice were randomly divided into seven groups designated; Group 1: Not infected, not treated - Control (C); Group 2: Infected, not treated (IU); Group 3: Infected, treated with chloroquine (IC); Group 4: Infected, treated with lumefantrine-trimethoprim-copper complex (ILTC); Group 5: Infected, treated with trimethoprim (IT); Group 6: Infected, treated with lumefantrine (IL); Group 7: Not infected, treated with lumefantrine-trimethoprim-copper complex (NILTC).

The mice in groups 1 and 2 were administered 0.2 ml of distilled water. Group 3 was administered 0.2 ml of chloroquine equivalent to 5.64mg/kg body weight, while the last four groups were administered 0.2ml of each drug corresponding to 4.56mg/kg body weight for a period of 3 days respectively. All the animals were sacrificed 24 hours after the completion of their doses.

Curative test

The curative test was carried out using a modified method of Riley and Peters' suppression test (Ryley and Peters 1970). The experimental mice were passage with standard inoculums of rodent *Plasmodium berghei*. Following 72 hours post infection, establishment of malaria parasite was confirmed and then a 3-day treatment commenced. Preparation of slides started 24hr after the first treatment.

Estimation of percentage parasitaemia in experimental mice

Percentage parasitaemia was estimated at the end of the 3 days treatment by counting the number of Parasitized red blood cells (PRBC) seen in 10^3 Red blood cell (RBC) using the x100 oil immersion microscope. Percentage parasitaemia was then calculated as follows:

$$\% \text{ Parasitaemia} = \frac{\text{Total number of PRBC}}{\text{Total number of RBC}} \times 100$$

Estimation of percentage chemosuppression

The percentage chemosuppression of parasite multiplication was calculated using the formula (Dikasso *et al.*, 2006).

$$\%Suppression = \frac{\text{Parasitaemia in negative control - Parasitaemia in study group}}{\text{Parasitaemia in negative control}} \times 100$$

Enzyme assays

The method described by Wright and Plummer (1974) was employed to assay for ALP activity. The method of Reitman and Frankel (1957) was used to determine the activities of AST and ALT. The LDH activity was measured using the method of Kubowitz and Ott (1943) as modified by Wroblewski and La Due (1955).

Assay for plasma electrolytes

The concentration of plasma sodium and potassium were spectrophotometrically determined as described in the Teco ® diagnostic kit manual. Plasma concentrations of chloride and bicarbonate ions were measured using mercuric nitrate method and back titration method respectively described by Tietz (1995).

Determination of plasma creatinine and urea concentration

Plasma creatinine concentration was determined spectrophotometrically by the method described by Kaplan and Pesce (1989) while that of urea was determined spectrophotometrically using the procedure in Randox kit manual.

Haematological studies

Red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (HGB) level, white blood cells (WBC) count, mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were measured using the methods described by Adewuyi and Olatunji (1995).

DNA damage and chromosomal aberration analysis

The analysis of Chromosomal aberration was done using the method described by Bakare *et al.* (2009). Kidney and liver samples of animals were homogenized in 5ml of 0.56% KCl and left at room temperature for 30 minutes. The cell suspension was then overlaid with 1ml freshly prepared chilled carnoy's fixative, mixed with a vortex mixer and centrifuged at 2,000 rpm for 10 minutes. The supernatant was pipetted into clean tubes and overlaid with 0.5ml freshly prepared chilled carnoy's fixative. The tubes were kept at 4^0C for 30 minutes for thorough fixation.

The contents were gently mixed and centrifuged again at 2,000 rpm for 10 minutes, supernatant was separated and fresh fixative was added. Fixing and centrifuging was done twice until a clear transparent cell suspension was obtained. A drop or two (little quantity) of the clear cell suspension was put on microscopic slides, air dried and stained with 5% Giemsa stain for 20 minutes. The slides were made permanent by dipping them in xylene after which drops of Canada balsam was added and then they were covered with cover slips. Metaphase spreads were observed at a magnification of x100 and analysed.

Statistical Analysis

All data are expressed as means of 5 replicates. Statistical analyses were carried out using ANOVA and DUNCAN's multiple range tests. A comparison of data from test groups and controls was carried out and differences considered significant at *p*< 0.05.

RESULTS

Percentage parasitaemia in the mice administered chloroquine, LTC complex, trimethoprim, lumefantrine is shown in Table 1. The parasitaemia level of the IU group at day 1, 2 and 3 post inoculation was significantly higher (*p*< 0.05) than those treated with chloroquine or LTC complex. There was no significant difference in parasitaemia level in mice treated with trimethroprim and lumefantrin (*p* > 0.05) three days post inoculation. Decrease in the parasitaemia level was observed in all groups after each day, with the exception of the untreated group. ILTC group showed a more noteworthy reduction in parasite level than all other treated groups.

The percentage chemosuppression of the *P. berghei* multiplication achieved by LTC complex was higher than that of lumefantrine or trimethoprim at day 6 and 8. At day 4 and 8, the percentage chemosuppression attained by LTC complex was comparable to that of chloroquine and was even higher at day 6 (Table 2).

The effect of lumefantrine, trimethoprim, and LTC complex on the specific activity of AST, ALT, ALP and LDH in the liver of *P. berghei* NK-65 infected mice is presented in Figure 1. The activity of AST, ALT, and ALP in the liver of control group was significantly higher (*P*< 0.05) than all the other groups observed. In contrast, LDH specific activity in the liver of control group was significantly reduced when compared with the activities observed in all the treated groups. There was no significant difference in liver activity of AST, ALT, ALP and LDH (*P* > 0.05) between ILTC group and NILTC group. Similarly, there was also no significant difference in liver LDH activity between IT group and IL group.

Figure 2 highlights the effect of lumefantrine, trimethoprim, and LTC complex on the specific activity of AST, ALT, ALP and LDH in the plasma of *P. berghei* NK-65 infected mice. In the plasma, there was significant increase (*P*< 0.05) in the activity of AST, ALT, and ALP across all groups when compared with the control. However, the plasma activity of LDH in the treated groups was significantly higher than the activity observed in the control. There was no significant difference in

Table 1: Percentage parasitaemia in *P. bergei* infected mice treated with chloroquine, lumefantrine, trimethoprim, and lumefantrine-trimethoprim-copper complex

| Group	Day 1 (%)	Day 2 (%)	Day 3 (%)
IU	31.89 ± 0.57^b	44.09 ± 0.75^c	53.59 ± 0.95^c
IC	29.62 ± 0.12^a	22.62 ± 0.10^a	19.42 ± 0.03^a
ILTC	29.43 ± 0.12^a	24.39 ± 0.09^a	18.39 ± 0.03^a
IT	30.17 ± 0.25^b	26.17 ± 0.23^b	21.04 ± 0.22^b
IL	30.22 ± 0.30^b	26.84 ± 0.29^b	21.17 ± 0.23^b

Each value is a mean of five replicates ± SEM. Rows with different superscripts are significantly different (P<0.05). IU - Infected untreated, IC – Infected treated with chloroquine, ILTC – Infected treated with lumefantrine–trimethoprim –copper complex, IT - Infected treated with trimethoprim, IL – Infected treated with lumefantrine.

Table 2: Percentage chemosuppression of parasite in *P. berghei* infected mice treated with chloroquine, lumefantrine, trimethoprim, and lumefantrine-trimethoprim-copper complex.

	%CHEMOSUPPRESSION			
	IC	ILTC	IT	IL
DAY 4	78.94	78.94	56.14	47.37
DAY 6	86.67	88.00	69.33	61.33
DAY 8	96.84	96.84	76.84	75.79

Each value is a mean of five replicates. IU - Infected untreated, IC – Infected treated with chloroquine, ILTC – Infected treated with lumefantrine–trimethoprim –copper complex, IT - Infected treated with trimethoprim, IL – Infected treated with lumefantrine.
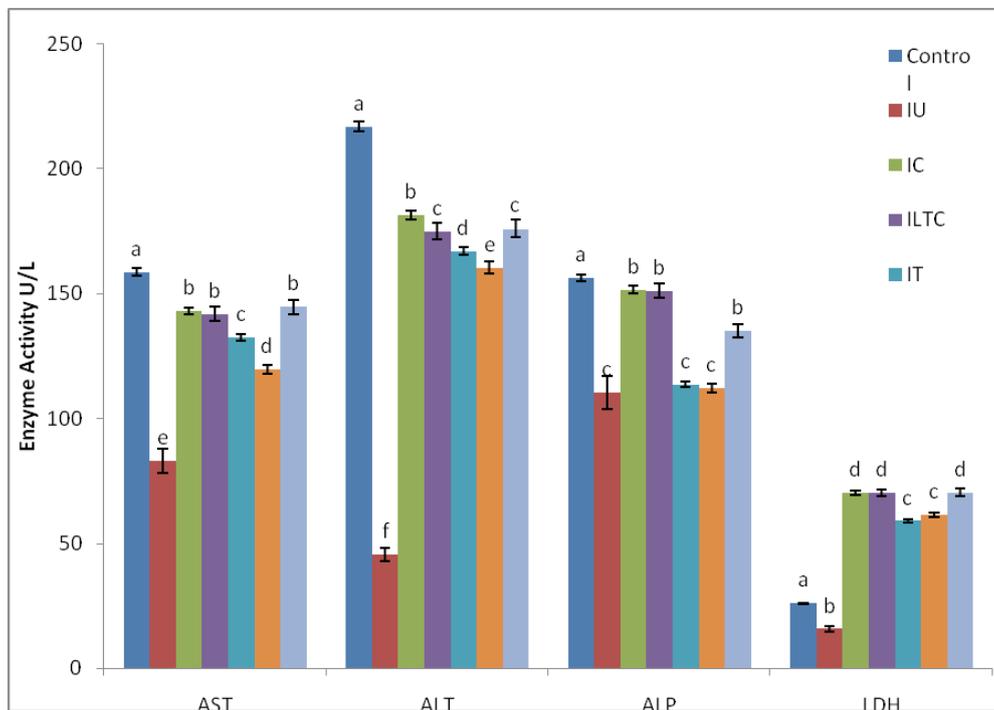
Figure 1: The effect of lumefantrine, trimethoprim, and lumefantrine - trimethoprim- copper complex on the activity of AST, ALT, ALP and LDH in the liver of *P. berghei* NK-65 infected mice. Values are mean of 5 replicates ± SEM. Bars with different superscript are significantly (p < 0.05) different.
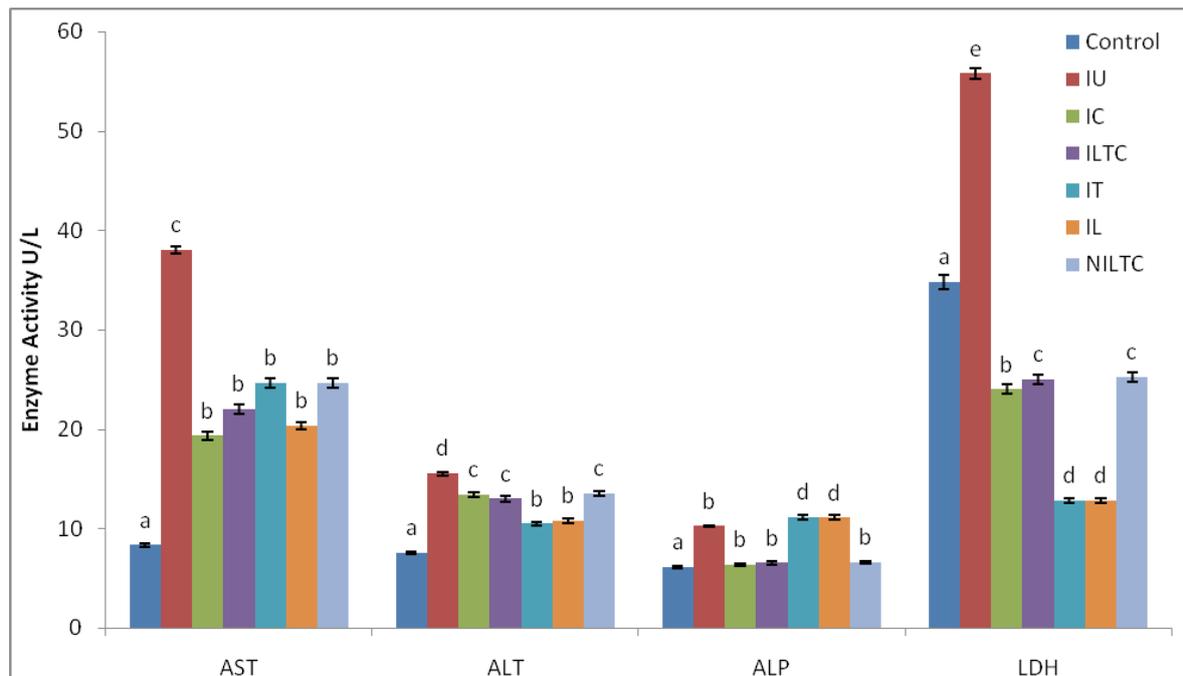
Figure 2: The effect of lumefantrine, trimethoprim, and lumefantrine-trimethoprim-copper complex on the activity of AST, ALT, ALP and LDH in the plasma of *P. berghei* NK-65 infected mice. Values are mean of 5 replicates ± SEM. Bars with different superscript are significantly (p < 0.05) different.

plasma activity of AST, ALT, ALP and LDH ($P > 0.05$) between ILTC group and NILTC group. Also, there was no significant difference in plasma LDH activity between IT and IL groups.

The effects of lumefantrine, trimethoprim and LTC complex on biochemical parameters in *P. berghei* NK-65 infected mice are shown in Table 3. There was a significant decrease ($P < 0.05$) in plasma potassium ion concentration across the groups when compared with the control, although there was no significant difference between the control and the NILTC group. There was no significant difference between the groups treated with IL and IT groups. Similarly, there was a significant decrease in the concentration of sodium ion across all the groups when compared with the control except for the IC and ILTC groups which were not significantly ($P > 0.05$) different from the control group. The concentration of plasma chloride ion also decreased across the group, although the concentration of chloride ion in the NILTC group was not significantly different from that of the control. In the same vein, there was a significant decrease in the concentration of bicarbonate ion across the groups studied when compared with the control. On the other hand, the plasma concentration of creatinine increased across the group compared with the control. The concentration of urea in the plasma of the IC and ILTC groups was significantly lower than the control and other groups. There was a significant increase in the concentration of total protein in the plasma across the groups compared with the control, except for the group NILTC which compared favourably with the control.

Table 4 shows the effects of lumefantrine, trimethoprim and LTC complex on haematological parameters in *P. berghei* NK-65 infected and non-infected mice. There

was a significant decrease in the levels of HGB, WBC, haematocrit (HCT), RBC, platelet (PLT) compared with the control across all the infected but treated groups. Contrarily, there was an increase in the levels of MCV, MCH, MCHC, and lymphocytes across all the groups when compared with the control.

Table 5 and 6 show the effects of lumefantrine, trimethoprim and LTC complex on chromosomal aberration in the liver and kidney of *P.berghei* NK-65 infected mice, respectively. There was a significant decrease ($P < 0.05$) in the total number of dividing cells, mitotic index in the liver and kidney across all the groups studied compared with the control, except for the IU group where there was no significant difference when compared with the control. There was a significant increase in the percentage chromosome aberration across all the groups compared with the control although the percentage chromosome aberration of the IU group was not significantly different from the control.

Table 3: Effects of administration of lumefantrine, trimethoprim and lumefantrine trimethoprim-copper complex on biochemical parameters in *P.berghei* NK-65 infected and non-infected mice.

Groups	K^+	Na^+	Cl^-	$HCO3^-$	Creatinine	Urea	Total Protein
C	3.88±0.03[e]	89.00±17.40[c]	125.2±0.21[e]	25.41±0.10[c]	2.25±0.17[a]	17.80±2.35[b]	6.21±0.03[a]
IU	2.57±0.01[c]	68.00±6.10[bc]	62.61±1.31[b]	17.88±0.44[a]	28.86±0.06[d]	20.49±3.70[b]	15.27±0.08[d]
IC	2.43±0.01[b]	81.00±12.00[c]	43.63±2.18[a]	19.55±0.76[ab]	36.14±0.08[e]	9.07±0.01[a]	11.57±0.53[c]
ILTC	3.07±0.03[d]	74.00±18.7[c]	42.43±1.64[a]	18.04±0.50[a]	12.00±2.36[b]	8.06±0.01[a]	9.91±0.03[b]
IT	1.737±0.03[a]	14.00±5.29[a]	94.58±6.46[c]	21.56±1.00[b]	12.13±2.43[b]	35.28±1.33[d]	11.85±0.60[c]
IL	1.700±0.02[a]	38.0±6.08[ab]	107.5±3.75[d]	19.55±0.95[ab]	21.84±0.09[c]	29.23±0.01[c]	11.72±0.20[c]
NILTC	3.89±0.04[e]	14.00±1.00[a]	153.33±1.04[f]	21.37±0.55[b]	14.56±0.02[b]	29.56±0.34[c]	6.67±0.86[a]

Values are expressed as mean of five replicates ± S.E.M and values in each row with different superscripts are significantly different ($p<0.05$).IU - Infected untreated, IC – Infected treated with chloroquine, ILTC – Infected treated with lumefantrine–trimethoprim –copper complex, IT - Infected treated with trimethoprim, IL – Infected treated with lumefantrine.

Table 4: Effects of lumefantrine, trimethoprim and lumefantrine-trimethoprim-copper complex on haematological parameters in *P. berghei* NK-65 infected and non-infected mice.

GROUP	CONTROL	IU	IC	ILTC	IT	IL	NILTC
RBC	7.49±0.41[d]	4.80±0.066[a]	6.21±0.11[c]	5.93±0.26[bc]	5.2±0.14[ab]	5.51±0.45[ab]	7.13±0.18[d]
WBC	16.06±0.03[f]	2.13±0.09[a]	9.72±0.40[d]	8.06±0.09[c]	7.55±0.10[c]	4.80±0.37[b]	15.26±0.40[e]
HGB	10.37±1.13[c]	4.63±1.93[a]	8.40±0.42[b]	7.11±0.02[ab]	8.36±0.42[bc]	8.53±0.32[bc]	9.82±0.19[bc]
HCT	36.27±1.16[d]	23.13±1.79[a]	29.43±0.51[c]	28.66±0.39[d]	26.89±0.39[bc]	24.59±0.09[ab]	35.53±1.11[d]
MCV	46.67±0.89[a]	50.27±2.83[a]	48.81±0.83[a]	49.08±2.21[a]	47.23±1.18[a]	46.87±0.83[a]	45.57±1.82[a]
MCH	13.39±0.31[a]	14.69±0.69[a]	14.63±0.32[a]	13.73±0.65[a]	13.28±0.42[a]	13.59±0.32[a]	13.97±0.52[a]
MCHC	28.56±1.02[ab]	29.32±0.91[ab]	31.12±1.53[b]	26.96±0.15[a]	28.23±1.31[ab]	28.17±1.31[ab]	30.11±0.95[ab]
PLT	694±3.75[f]	318±9.07[a]	578±1.62[e]	507±5.00[d]	343±4.67[b]	423±4.93[c]	591±2.89[e]
LYM	41.83±1.11[a]	64.92±0.68[cd]	50.89±1.62[ab]	70.34±1.81[d]	85.33±5.24[e]	55.97±3.56[bc]	88.67±4.41[e]

Values are expressed as mean of five replicates ± S.E.M and values in each row with different superscripts are significantly different ($p<0.05$).IU - Infected untreated, IC – Infected treated with chloroquine, ILTC – Infected treated with lumefantrine–trimethoprim –copper complex, IT - Infected treated with trimethoprim, IL – Infected treated with lumefantrine.

Table 5: Effects of administration of lumefantrine, trimethoprim and lumefantrine-trimethoprim-copper complex on the metaphasic status of chromosomes of the liver of *P. berghei* NK-65 infected mice.

Groups	TNDA	%CA	MI
C	1433 ± 1.20[c]	0.00 ± 0.00[a]	47.78 ± 4.00[c]
IU	1481.7 ± 1.83[c]	0.135 ± 0.04[a]	49.39 ± 0.61[c]
IC	1381.3 ± 9.21[c]	2.50 ± 0.26[c]	46.04 ± 3.07[c]
ILTC	918 ± 1.42[b]	1.364 ± 0.31[b]	30.6 ± 4.72[b]
IT	833 ± 6.66[b]	3.78 ± 0.17[d]	27.78 ± 2.22[b]
IL	800 ± 6.00[ab]	2.92 ± 0.65[cd]	26.66 ± 0.02[ab]
NILTC	551 ± 2.89[a]	2.69 ± 0.36[c]	18.38 ± 0.96[a]

Values are expressed as mean of five replicates ± S.E.M and values in each row with different superscripts are significantly different (p<0.05).IU - Infected untreated, IC – Infected treated with chloroquine, ILTC – Infected treated with lumefantrine–trimethoprim – copper complex, IT - Infected treated with trimethoprim, IL – Infected treated with lumefantrine.

Table 6: Effects of administration of lumefantrine, trimethoprim and lumefantrine-trimethoprim-copper complex on the metaphasic status of chromosomes of the kidney of *P. berghei* NK-65 infected mice.

Groups	TDNA	%CA	M.I
C	1560 ± 3.05[d]	0.00 ± 0.00[a]	52 ± 1.02[d]
IU	1495 ± 3.04[d]	0.468 ± 0.03[a]	49.83 ± 1.01[d]
IC	966.67 ± 3.33[c]	3.141 ± 0.07[c]	32.22 ± 1.11[c]
ILTC	766.67 ± 3.33[b]	1.845 ± 0.23[b]	25.56 ± 1.11[b]
IT	844.33 ± 7.28[bc]	3.41 ± 0.30[c]	28.14 ± 2.43[b]
IL	844 ± 4.43[bc]	1.602 ± 0.542[b]	28.14 ± 1.48[b]
NILTC	582.67 ± 1.26[a]	2.279 ± 0.267[b]	19.42 ± 0.42[a]

Values are expressed as mean of five replicates ± S.E.M and values in each row with different superscripts are significantly different (p<0.05).IU - Infected untreated, IC – Infected treated with chloroquine, ILTC – Infected treated with lumefantrine–trimethoprim – copper complex, IT - Infected treated with trimethoprim, IL – Infected treated with lumefantrine.

DISCUSSION

This *in vivo* antimalarial study revealed that LTC complex has a better parasite clearance effect than pure lumefantrine or trimethoprim and also compared favourably with chloroquine. This indicates that the coordination of copper to the ligands (lumefantrine and trimethoprim) enhances the antiplasmodial activity of the drug. Rajapakse *et al.* (2009) also reported similar observation in which Ruthenium (II) chloroquine complexes were very effective against chloroquine resistant strains of *P. falciparum*. Metal compounds are usually appropriate for the treatment of parasitic diseases such as malaria because they display a distinct selectivity for some parasites' biomolecules compared to the host's biomolecules (Navarro *et al.,* 2010).

Decreased activities of AST and ALT in the liver and increased activities in the plasma of the group treated with LTC compared with that of the control, suggests leakage of the enzymes from the liver into the plasma. Increased

efflux of these enzymes into the plasma might be as a result of stress inflicted on the tissue by the drug administered leading to plasma membrane disruption. Similar observation was reported in our previous studies (Arise *et al.,* 2016, 2017). Increased plasma activities of ALP observed in the treated groups may be due to disruption of plasma membrane of the hepatocytes since the liver activities of ALP in the treated groups were significantly reduced when compared with the control. LDH is present in almost all tissues in the body and plays a key role in cellular respiration (production of usable energy from glucose). Hepatic increase in the activity of the enzyme compared to the control may be due to the induction of the enzyme perhaps by *de novo* synthesis (Malomo *et al.,* 1995).

Renal function tests are usually used to study the presence of active laceration in the kidney, or to examine the proper functioning of the different parts of the nephron. Urea and creatinine are important metabolic products of protein

catabolism and muscle metabolism respectively and are used in the measurement of glomerular filtration rate (Arise *et al.* 2016). The detected difference in the concentrations of urea and creatinine in the plasma compared with that of the control indicates that the drugs may have impaired the clearance function of the kidney. This corresponds with our previous work where the antimalarial drugs impaired the clearance function of the kidney (Arise *et al.*, 2016). The resting membrane electrical potential is mainly determined by potassium ion and it plays a vital role in cellular metabolism, particularly in the synthesis of protein and glycogen as well as enzymatic processes essential for cellular energy. In a state of dysfunction, there is a slow but continuous leakage of potassium ion from the cells into the immediate plasma along a concentration gradient as a result of sodium potassium ATPase pump failure (Opoku-Okrah *et al.,* 2015). Hence, the observed difference in the concentration of K^+ might be as a result of alteration in the ATPase pump, disrupting the ratio of intracellular to extracellular potassium. Sodium is the major electrolyte in the extracellular fluid, an alteration in its concentration and that of bicarbonate ions in the plasma may indicate the occurrence of glomerular dysfunction (Tietz *et al.,* 1994). Chloride ion is predominantly extracellular anion and its concentration is about 26 times more than its intracellular concentration. Chloride ion plays a key role in maintaining the pH of the extracellular fluids. The significant decrease in the concentrations of chloride and bicarbonate ions in the blood caused by the drugs, could lead to disturbance in acid-base balance which is inclined to offset metabolic alkalosis (Abubakar and Sule, 2010).

The largest amount of the total solutes present in the plasma is constituted by total protein. The amounts of serum total protein (transferrin, albumin, and prealbumin) are often included in the liver function tests. Increased levels of immunoglobulin have been linked with some chronic liver diseases, which in turn increase total protein. Total serum protein level elevation may be as a result of leakage of tissue specific enzymes and other intracellular proteins, due to cell membrane disruption induced by the drugs (Orhue *et al.,* 2005).

Changes in haematological parameters are some of the major common complications in malaria and they play a key role in the pathogenesis of malaria. These changes occur in major cell types such as RBCs, leucocytes and thrombocytes (Bakhubaira 2013; Maina *et al.,* 2010). Malaria parasites invade and remodel human red blood cells (RBCs) by trafficking parasite-synthesized proteins to the RBC surface, thereby affecting their function and contributing to the pathogenesis (Birch *et al.,* 2015). It was hypothesized that a key step in the aetiology of severe malaria is the sequestration of parasite-infected RBCs within the microvasculature of the host organism, partly due to molecular interaction from the parasite derived ligands (Miller *et al.,* 2013; Haldar *et al.,* 2007). RBC, HGB, WBC, PLT, HCT, in the groups treated with the drugs were significantly higher than the infected but untreated group, however, they were significantly lower than the control. This is similar to the observation of Kotepui *et al.* (2014) that reported that RBC, haemoglobin, platelets,

WBC, neutrophil, monocyte, lymphocyte and eosinophil counts were significantly lower in malaria-infected patients; while mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil-lymphocyte ratio (NLR), mean corpuscular volume (MCV), and monocyte-lymphocyte ratio (MLR) were significantly higher when compared with non-malaria infected subjects. The inability of the drug to increase the counts of the haematological parameters comparable to the control levels is similar to the report of Esan (2014), where WBC count in post anti-malarial drug treatment was observed to be lower compared to pre-anti-malarial drug treatment. Similar to this present study, Adjuik *et al.* (2002) reported a general trend of slight decrease in both total WBCs and neutrophil counts after anti-malarial drug treatment. The lowered number of RBCs in the treated groups may indicate that the drug lacked erythropoietic potentials, predisposing to anaemia. Also, the oxygen carrying capacity of the blood and the amount of oxygen delivered to the tissues may have been reduced as a result of the drug administration (Arise *et al.,* 2016).

The reduction in the total number of dividing cells and mitotic index in the liver and kidney after the administration of LTC complex indicates that the drug may be cytotoxic, and thereby cause the inhibition of mitotic activities (Anjana and Thoppil, 2013). The observed reduction in mitotic activity and presence of chromosomal aberration may be due to the blockage of DNA synthesis (Musanovic *et al.,* 2013). Antimalarial drugs have been reported to induce some deleterious effects including reduction of mitotic index by mefloquine (El-Habit and Al-Khamash, 2013), and inhibition of DNA synthesis by chloroquine (Nwangburuka and Oyelana, 2011).

CONCLUSION

The antimalarial activity of LTC complex investigated using mice infected with *Plasmodium berghei* shows that the complex has higher antimalarial activity than pure lumefantrine or trimethoprim, and also compared favourably with chloroquine. The observed chromosomal aberration and altered biochemical indices suggest functional and selective toxicity of the drug.

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