**Original Article**

# Solvent-less Synthesis, Antimalarial and Toxicity Evaluation of Lumefantrine-Copper Complex in Swiss Mice

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**Abstract**

The antimalarial efficacy and safety of mechanically induced solventlessly synthesized lumefantrine-copper complex were investigated in experimental mice. Parasite level in *Plasmodium berghei*-infected mice treated with lumefantrine - copper complex (LCC) significantly declined ($p < 0.05$) at day 3 and was comparable with that of chloroquine-treated mice. LCC attained a percentage chemo-suppression which was significantly higher than those of pure lumefantrine and comparable with chloroquine. Pure lumefantrine attained a clearance of 88.52%, chloroquine was 91.95%, while LCC was 95.10%. Administration of lumefantrine, LCC and chloroquine to mice for 7 days caused a significant increase ($p < 0.05$) in the activities of alkaline phosphatase, acid phosphatase and lactate dehydrogenase in the liver when compared with the control, and a significant reduction ($p < 0.05$) in the liver and kidney activities of alanine and aspartate aminotransferases when compared with the control. Also, there was a significant decrease ($p < 0.05$) in the levels of PCV, Hb, RBC and lymphocytes and a significant increase ($p < 0.05$) in the white blood cells count and neutrophil counts in all the treatment groups when compared with control. Alterations in the biochemical parameters and chromosomal aberration in the organs investigated suggested selective, chromosomal and functional toxicity of the tested drugs.

**Keywords:** chloroquine, chromosomal aberration, lumefantrine-copper complex, malaria, solvent-less synthesis

**Abbreviations:** ALT - alanine aminotransferase, AST - aspartate aminotransferase, b.wt. - body weight, LCC – lumefantrine - copper complex, PCV - packed cell volume, Hb - haemoglobin level, WBC - white blood cells count, RBC - red blood cell count, MCV - mean corpuscular volume, MCH - mean corpuscular, TNMA – total number of metaphases analysed, MCA – metaphases with chromosomal aberration, MI – mitotic index, CA – chromosomal aberration

## Introduction

Studies have shown that in Africa, Nigeria has one-fourth of all malaria cases (WHO, 2014). All through the year, transmission occurs in Southern Nigeria, while it is more seasonal in the North. Malaria, a life-threatening disease, occurs as a result of bites of infected female mosquitoes. About 214 million cases were reported in 2015, while there was about 37% global decrease in malaria incidence between 2000 and 2015 (WHO, 2015). It is caused by the parasitic unicellular protozoans that belong to the genus *Plasmodium*. Usually, the disease is transmitted through a bite from an infected female *Anopheles* mosquito, which introduces the parasite into the human circulatory system (WHO, 2014).

The menace of malaria has resulted in a reduced economic development in African countries by 1.3% per year (Asenso-Okyere *et al.*, 2011). Due to the accumulated effects of over 35 years, the gross domestic product of African countries, particularly Nigeria, is now approximately 32% lower than it

would be expected to be without malaria (WHO, 2013). 85–90% of malaria fatalities are found in sub-Saharan Africa (Layne, 2006). Ivory Coast (86.15%), Angola (56.93%) and Burkina Faso (50.66%) were estimated in 2009 to have the highest death rate per 100,000. In 2010, a study estimated that Burkina Faso, Mozambique and Mali had the deadliest cases of malaria (Murray *et al.*, 2012).

Oral medications are available for the therapy of uncomplicated malaria (WHO, 2015). The use of artemisinins in conjunction with other antimalarial drugs (in a therapeutic approach referred to as artemisinin-combination therapy, ACT) is the most efficient therapeutic approach for *P. falciparum* infection, which also results in reduced resistance to any single drug component. Examples of these additional antimalarials are: lumefantrine, amodiaquine, sulfadoxine / pyrimethamine or mefloquine (WHO, 2001). The incessant spread of resistance of *P. falciparum* to antimalarial drugs presents a menace to malaria control plans (Adebayo and Krettli, 2011).

Lumefantrine is an antimalarial drug used to treat acute uncomplicated malaria. It is administered in a combination therapy in conjunction with artemether, for enhanced effectiveness (Omari *et al.*, 2003). The mechanism of action of the combination therapy involves activity against the erythrocytic stages of *Plasmodium* spp. and is thus employed for the treatment of *P. falciparum* infections and unidentified *Plasmodium* species, including infections acquired in chloroquine-resistant areas (Omari *et al.*, 2006). Lumefantrine, an aryl-amino alcohol (Aweeka and German, 2008), prevents detoxification of heme. Toxic heme and free radicals bring the death of malaria parasite (Kokwaro *et al.*, 2007).

As a result of environmental pollution, related to solvent disposals and reduced sources of energy supply, it was required the developing of benign synthetic pathways which are simple and exhibit high atom economy (Cave *et al.*, 2001). Mechanochemical synthesis also referred to as solvent-free approach ranges from merely grinding reactants using a mortar and pestle, to ball milling procedure (Tella *et al.*, 2011). Previous studies have shown that the effectiveness of some therapeutic agents was enhanced upon coordination to transition metals (Ogunniran *et al.*, 2007; Tella *et al.*, 2015). Incorporation of metallocene into each of quinine and chloroquine has been reported to produce compounds that exhibited activity against both *Plasmodium* chloroquine-sensitive and chloroquine-resistant strains (Obaleye *et al.*, 2009) and were reported to be safe and non-mutagenic in mice (Dormale *et al.*, 1998).

In continuation of the authors' efforts to find new antimalarial drugs effective against chloroquine resistant strain of the malaria parasite, this work seeks to make known the biological activities (antimalarial effect and safety) of lumefantrine-copper complex.

## Materials and Methods

### Drugs used

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

### Experimental animals

Thirty white male albino mice (*Mus muculus*) with an average weight of $16.79 \pm 0.36$ 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* (chloroquine sensitive NK-65) was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria.

### Assay kits and reagents used

Lactate dehydrogenase, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase assay kits were products of Randox Laboratories Ltd., United Kingdom. All other reagents used were of analytical grade and were prepared in all glass apparatus using distilled water.

### Mechanochemical synthesis of lumefantrine-copper complex

A stoichiometric 1:1 mixture of copper II chloride salt (1 mole, 0.170 g) and lumefantrine (1 mole, 0.450 g) was manually grounded in an agate mortar which had been washed and cleaned before use (Fig. 1). The reactants were pulverized for 15 minutes to yield a yellow substance. Drops (2) of methanol were added to make a more homogenous complex resulting in a pale green complex. Evolution of gases and colour changes were observed. The powder was collected for melting point, infrared spectra and X-ray Powder Diffraction (XPRD) analyses.

### Inoculation of experimental mice

Each mouse in the infected groups (20 mice, 5 per group) was inoculated intraperitoneally with 0.2 ml of infected blood containing about $1 \times 10^7$ *P. berghei* parasitized red blood cells. Blood smear was prepared, stained and observed under the microscope to establish parasitaemia on the fifth day after inoculation (Ryley and Peters, 1970).

### Experimental design

Anti-malarial study was investigated in mice. The rats were supplied with normal rat chow and water *ad libitum*. The experimental animals were randomly divided into six groups as follows:

Group 1: not treated, not infected - Control (C);
Group 2: infected but not treated (IU);
Group 3: infected and treated with chloroquine (IC);
Group 4: infected and treated with lumenfantrine (IL);
Group 5: infected and treated with lumenfantrine-Cu (IC);
Group 6: Not infected but treated with lumenfantrine-Cu (NILC)

The mice in groups 1 and 2 were administered 0.2 ml of distilled water. Mice in group 3 were administered 0.2 ml of 5.5 mg/kg b.wt. chloroquine. Other groups were administered 0.2 ml of each drug corresponding to 1.71 mg/kg body weight for a period of 3 days respectively. All experimental animals were sacrificed 24 hours after their last doses.

### Curative tests

The design adopted was the method modified by Ryley and Peters (1970). This was carried out by passaging of the experimental mice with standard inoculums of rodent *P. berghei* and confirmation of establishment of malaria parasite infection 72 hours post-infection before the commencement of treatment.
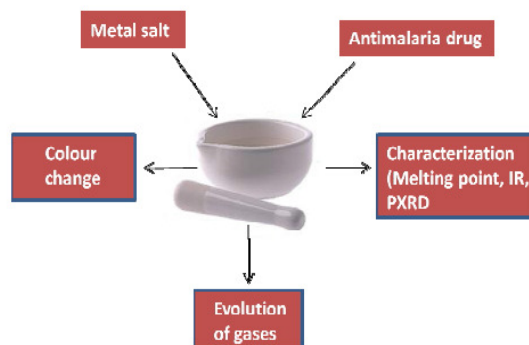


Fig. 1. Schematic diagram of mechanochemical synthesis (grinding)

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The preparation of slides started 24 hours after the first treatment.

### Toxicity evaluation
### Enzyme assays

The procedure described by Roy (1970) was used to assay alkaline phosphatase (ALP) activity. Aspartate aminotranferase (AST) and alanine aminotranferase (ALT) activities were assayed using the method of Reitman and Frankel (1957). Lactate dehydrogenase activity was measured following the method of Kubowitz and Ott (1943) as modified by Wroblewski and La Due (1955). Acid phosphatase activity was measured as described by Wright *et al.* (1972).

### Hematological parameters

Packed cell volume (PCV), haemoglobin level (Hb), white blood cells count (WBC), mean corpuscular haemoglobin concentration (MCHC) and platelet count were determined using the methods of Adewuyi and Olatunji (1995).

### DNA damage and chromosomal aberration evaluation

Chromosomal aberration analysis was carried out using the method described by Bakare (2009). Kidney and liver samples were homogenized in 5 ml of 0.56% KCl and left at room temperature (25 °C) for 30 minutes. The cell suspension was quickly overlaid with 1 ml freshly prepared chilled Carnoy's fixative and was gently mixed with a vortex mixer. Centrifugation was done at 2,000 rpm for 10 minutes. The supernatant was pipetted into a clean tube and slowly overlaid with 0.5 ml freshly prepared chilled Carnoy's fixative. The tubes were kept at 4 °C for 30 minutes through fixation; the contents were gently mixed and centrifuged at 2,000 rpm for 10 minutes. The supernatant was gently removed without disturbing the pellets and fresh fixative was added. The fixing and centrifuging was done twice until a clear transparent cell suspension was obtained. A pipette was used to drop a small quantity of the cell suspension onto grease free, pre-cleaned glass slides. The slides were air dried and stained with 5% Giemsa stain for 20 minutes. The slides were made permanent by dipping them in xylene and then drops from Canada balsam were added and covered with slips, metaphase spreads were observed at a magnification of 1000x.

### Statistical analysis

Data were expressed as means of 5 replicates. Data from the test groups were compared with their respective controls and differences were considered significant at $p < 0.05$. Statistical analysis was determined using ANOVA and DUNCAN's multiple range tests (Mahajan, 1997).

## Results

The complex synthesized had a yellow colour and a melting point of 115 °C. It was soluble in methanol and distilled water. Infrared spectra data also revealed that lumefantrine behaved as a ligand with donor sequence towards copper ions, with which coordination took place. The metal chloride salt reacted with the ligand "L" forming a complex using the proposed equation:

$$MX_2 + L = ML.X_2$$
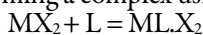
Where: M represents $Cu^{2+}$ metal salt; L, lumefantrine and X, Chloride ion.

Infrared spectra of lumefantrine–copper complex are shown in Fig. 2.

Table 1 shows parasitaemia levels in *P. berghei* NK-65 infected mice treated with chloroquine, pure lumefantrine and lumefantrine - copper complex. There was a significant increase ($p < 0.05$) in parasitaemia in the IU group at days 2 and 3 post-inoculation when compared to other groups. The parasite level in mice treated with lumefantrine - copper complex significantly declined ($p < 0.05$) on day 3 when compared to previous days and was significantly lower than the parasitaemia in animals of the untreated group. The percentage of parasitaemia reduction in animals treated with lumefantrine (pure and copper complex) groups was comparable with that of the standard drug (chloroquine).

Table 2 shows the percentage chemo-suppression of parasite multiplication in *P. berghei* NK-65-infected mice treated with chloroquine, pure lumefantrine and lumefantrine - copper complex. The percentage chemo-suppression attained by lumefantrine - copper complex was significantly higher ($p < 0.05$) than pure lumefantrine
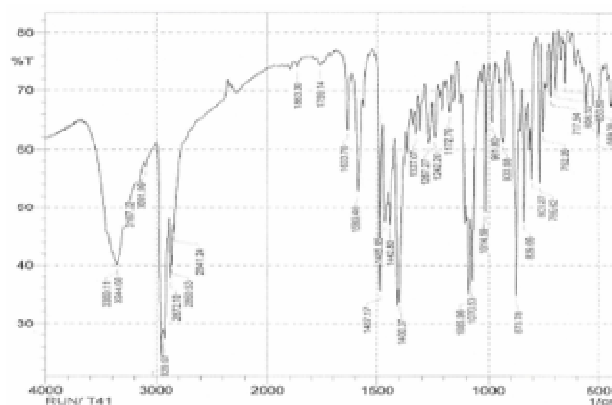


Fig. 2. Infrared spectra of lumefantrine - copper complex

Table 1. Parasitaemia levels in *P. berghei* NK-65 infected mice treated with chloroquine, pure lumefantrine and lumefantrine - copper complex

| Group | Day 1 (%) | Day 2 (%) | Day 3 (%) |
|-------|-----------|-----------|-----------|
| IU | 22.99 ± 0.72[a] | 34.40 ± 0.59[c] | 45.86 ± 0.82[b] |
| IC | 22.38 ± 0.43[a] | 15.60 ± 0.32[b] | 13.06 ± 0.62[a] |
| IL | 22.41 ± 0.64[a] | 18.84 ± 0.46[a] | 13.71 ± 0.61[a] |
| ILC | 22.37 ± 0.58[a] | 18.99 ± 0.59[a] | 12.32 ± 0.60[a] |

Each value is a mean of 5 replicates ± SEM. Rows with different superscripts were significantly different ($p < 0.05$); IU – Infected untreated, IC – Infected treated with chloroquine, IL – Infected treated with lumefantrine (pure), ILC- Infected treated with lumefantrine - copper complex

Table 2. Percentage chemo-suppression of parasite multiplication in *P. berghei* NK-65 infected mice treated with chloroquine, pure lumefantrine and lumefantrine - copper complex

| Group | Day 5 (%) | Day 7 (%) |
|-------|-----------|-----------|
| IC | 53.48 | 91.95 |
| IL | 45.23 | 88.52 |
| ILC | 47.70 | 95.10 |

Each value is a mean of 5 replicates; IC – Infected treated with chloroquine, IL – Infected treated with lumefantrine (pure), ILC- Infected treated with lumefantrine - copper complex

but comparable with chloroquine. Pure lumefantrine attained a clearance of 88.52%, chloroquine was 91.95%, while the lumefantrine - copper complex was 95.10%.

Table 3 shows the effect of lumefantrine and lumefantrine - copper complex on the specific activity of alkaline phosphatase (ALP) in the liver and plasma of *P. berghei* NK-65-infected mice. There was a significant reduction (p < 0.05) in the activity of ALP in the liver across all groups when compared with the control (treated with distilled water). Also, in the plasma there was a significant increase (p < 0.05) across all groups when compared with the control (treated with distilled water). Table 4 shows the effect of lumefantrine and lumefantrine - copper complex on the specific activity of aspartate aminotransferase (AST) in the liver and plasma of *P. berghei* NK-65-infected mice. There was a significant reduction (p < 0.05) in the activity of AST in the liver across all groups when compared with the control (group administered distilled water). However, in the plasma there was a significant increase (p < 0.05) across all groups when compared with the control (group administered distilled water).

Table 5 shows the effect of lumefantrine and lumefantrine - copper complex on the specific activity of alanine aminotransferase (ALT) in the liver and plasma of

*P. berghei* NK-65-infected mice. There was a significant decrease (p < 0.05) in the activity of ALT in the liver of lumefantrine and lumefantrine - copper complex-treated groups when compared with the control (treated with distilled water). However, in the plasma there was a significant increase (p < 0.05) in the activity of ALT between lumefantrine and lumefantrine - copper complex-treated groups when compared with the control (treated with distilled water) except in the negative control (infected untreated) and chloroquine-treated, which showed no significant difference (p > 0.05) in comparison with the positive control.

Table 6 shows the effect of lumefantrine and lumefantrine - copper complex on the specific activity of lactate dehydrogenase (LDH) in the liver and plasma of *P. berghei* NK-65 infected mice. There was a significant increase (p < 0.05) in the activity of LDH in the liver across all the groups except in the negative control where there was no significant difference when compared with the control (treated with distilled water). There was a significant increase (p < 0.05) in the plasma LDH activity across all groups when compared with the control (treated with distilled water) except in the group treated with chloroquine, which showed no significant difference (p > 0.05) in comparison with the positive control.

Table 3. The effect of lumefantrine and lumefantrine - copper complex on the specific activity of alkaline phosphatase (ALP) in the liver and plasma of *P. berghei* NK-65 infected mice

| Group | Liver ALP (U/L) | Plasma ALP (U/L) |
|---|---|---|
| C | 80.73 ± 7.06[c] | 7.36 ± 0.26[a] |
| IU | 59.08 ± 3.97[b] | 5.99 ± 0.42[a] |
| IC | 54.79 ± 3.08[a] | 9.79 ± 0.82[b] |
| IL | 60.63 ± 4.27[b] | 11.23 ± 1.01[c] |
| ILC | 65.27 ± 5.40[b] | 12.54 ± 1.41[c] |
| NLC | 54.77 ± 4.96[a] | 12.20 ± 1.42[c] |

Each value is a mean of 5 replicates ± SEM. Columns with different superscripts were significantly different (p < 0.05); C – Control, IU – Infected untreated, IC – Infected treated with chloroquine, IL – Infected treated with lumefantrine (pure), ILC- Infected treated with lumefantrine - copper complex

Table 5. The effect of lumefantrine and lumefantrine - copper complex on the specific activity of liver and plasma alanine aminotransferase of *P. berghei* NK-65 infected mice

| Group | Liver ALT (U/L) | Plasma ALT (U/L) |
|---|---|---|
| C | 127.98 ± 9.38[c] | 6.80 ± 0.54[a] |
| IU | 120.68 ± 10.12[c] | 7.89 ± 1.25[a] |
| IC | 125.90 ± 10.33[c] | 9.79 ± 2.51[b] |
| IL | 115.61 ± 11.19[b] | 14.09 ± 1.37[c] |
| ILC | 112.56 ± 9.56[b] | 16.48 ± 1.33[c] |
| NLC | 80.49 ± 7.89[a] | 16.29 ± 1.45[c] |

Each value is a mean of 5 replicates ± SEM. Columns with different superscripts were significantly different (p < 0.05); C – Control, IU – Infected untreated, IC – Infected treated with chloroquine, IL – Infected treated with lumefantrine (pure), ILC- Infected treated with lumefantrine - copper complex

Table 4. The effect of lumefantrine and lumefantrine - copper complex on the specific activity of aspartate aminotransferase in the liver and plasma of *P. berghei* NK-65 infected mice

| Group | Liver AST (U/L) | Plasma AST (U/L) |
|---|---|---|
| C | 154.97 ± 8.25[d] | 14.26 ± 1.45[a] |
| IU | 127.41 ± 6.14[c] | 18.60 ± 1.14[a] |
| IC | 121.37 ± 6.87[c] | 27.80 ± 1.78[b] |
| IL | 124.15 ± 5.65[c] | 31.85 ± 2.89[c] |
| ILC | 103.19 ± 5.71[b] | 35.11 ± 2.56[c] |
| NLC | 81.75 ± 4.65[a] | 41.35 ± 4.83[d] |

Each value is a mean of 5 replicates ± SEM. Columns with different superscripts were significantly different (p < 0.05); C – Control, IU – Infected untreated, IC – Infected treated with chloroquine, IL – Infected treated with lumefantrine (pure), ILC- Infected treated with lumefantrine - copper complex

Table 6. The effect of lumefantrine and lumefantrine - copper complex on the specific activity of lactate dehydrogenase (LDH) in the liver and plasma of *P. berghei* NK-65 infected mice

| Group | Liver LDH (U/L) | Plasma LDH (U/L) |
|---|---|---|
| C | 16.62 ± 1.15[a] | 12.78 ± 0.73[a] |
| IU | 26.26 ± 1.40[a] | 9.89 ± 0.40[a] |
| IC | 38.46 ± 2.70[b] | 18.64 ± 1.36[b] |
| IL | 58.42 ± 4.48[c] | 27.39 ± 1.78[c] |
| ILC | 82.45 ± 6.14[d] | 29.37 ± 1.26[c] |
| NLC | 50.99 ± 3.28[c] | 24.96 ± 1.07[c] |

Each value is a mean of 5 replicates ± SEM. Columns with different superscripts were significantly different (p < 0.05); C – Control, IU – Infected untreated, IC – Infected treated with chloroquine, IL – Infected treated with lumefantrine (pure), ILC- Infected treated with lumefantrine - copper complex

Table 7. The effect of lumefantrine and lumefantrine - copper complex on the concentration of some biochemical parameters in the plasma of *P. berghei* NK-65 infected mice

| Group | Na$^+$ | K$^+$ | Cl$^-$ | HCO$_3^-$ | Urea | Creatinine |
|---|---|---|---|---|---|---|
| C | 100.23 ± 2.33$^{bc}$ | 6.19 ± 0.36$^a$ | 74.88 ± 2.14$^a$ | 16.89 ± 0.77$^a$ | 9.36 ± 0.54$^b$ | 12.82 ± 3.26$^{ab}$ |
| IU | 92.01 ± 5.05$^b$ | 5.48 ± 0.37$^a$ | 79.26 ± 4.05$^a$ | 21.15 ± 4.05$^b$ | 6.57 ± 0.13$^a$ | 9.72 ± 0.90$^a$ |
| IC | 126.68 ± 2.08$^d$ | 5.64 ± 0.35$^a$ | 93.94 ± 1.79$^c$ | 16.70 ± 1.61$^a$ | 7.38 ± 0.46$^{ab}$ | 18.20 ± 4.39$^b$ |
| IL | 97.66 ± 4.16$^{bc}$ | 5.17 ± 0.71$^a$ | 91.38 ± 3.42$^{bc}$ | 21.22 ± 1.78$^b$ | 5.71 ± 1.00$^a$ | 18.41 ± 1.94$^b$ |
| ILC | 103.56 ± 2.85$^c$ | 10.90 ± 1.20$^b$ | 83.33 ± 4.25$^a$ | 18.17 ± 3.43$^{ab}$ | 6.11 ± 0.85$^a$ | 11.17 ± 2.45$^{ab}$ |
| NLC | 80.04 ± 2.52$^a$ | 12.19 ± 2.32$^b$ | 91.17 ± 1.22$^{bc}$ | 12.54 ± 0.81$^a$ | 6.32 ± 0.82$^a$ | 14.08 ± 0.40$^{ab}$ |

Each value is a mean of 5 replicates ± SEM. Columns with different superscripts were significantly different (p < 0.05). C – Control, IU – Infected untreated, IC – Infected treated with chloroquine, IL – Infected treated with lumefantrine (pure), ILC- Infected treated with lumefantrine - copper complex

Table 7 shows the effect of lumefantrine and lumefantrine - copper complex on the concentration of some biochemical parameters in the plasma of *P. berghei* NK-65-infected mice. Plasma urea concentration showed a significant decrease (p < 0.05) in all the groups except chloroquine-treated group which showed no significant difference (p > 0.05) when compared with the positive control. There was no significant difference (p > 0.05) in the concentration of plasma creatinine in each of the groups when compared with the positive control. However, the concentration of plasma creatinine in the groups administered with chloroquine and pure lumefantrine was significantly higher (p < 0.05) than the untreated ones, whereas there was no significant difference (p > 0.05) in the concentration of plasma creatinine in the group treated with lumefantrine (pure and copper-complexed) when compared with the chloroquine-treated group and the positive control.

The concentration of sodium ion in the plasma did not show any significant alteration (p > 0.05) in the group administered with lumefantrine (pure and copper-complexed) when compared with the positive control. However, there was a significant increase (p < 0.05) in the plasma sodium ion concentration of the groups administered with chloroquine. In contrast, there was no significant difference (p > 0.05) in the plasma concentration of potassium ion across the groups except for the significant elevation (p < 0.05) in the groups administered with lumefantrine - copper complex. There was a significant increase (p < 0.05) in the concentration of bicarbonate ion in the untreated group and the group administered with pure lumefantrine when compared with the positive control. The concentration of chloride ion in the plasma was significantly increased (p < 0.05) in each of the groups administered with chloroquine and pure lumefantrine when compared with the control, but no significant difference (p > 0.05) was observed in the group treated with lumefantrine - copper complex and chloroquine.

Table 8 shows the effect of lumefantrine and lumefantrine - copper complex on the concentration of some haematological parameters in the plasma of *P. berghei* NK-65 infected mice. There was a significant decrease (p < 0.05) in the levels of red blood cells, haemoglobin, PCV, lymphocyte, MCHC and MCH across all groups when compared with the positive control.

Table 8. Effect of lumefantrine and lumefantrine - copper complex on the concentration of some haematological parameters in the plasma of *P. berghei* NK-65 infected mice

| Parameter | C | IU | IC | IL | ILC | NLC |
|---|---|---|---|---|---|---|
| WBC | 5.84 ± 0.69$^a$ | 22.2 ± 2.75$^b$ | 21.62 ± 4.68$^b$ | 21.06 ± 1.28$^b$ | 19.40 ± 1.82$^b$ | 6.04 ± 0.62$^a$ |
| RBC | 8.60 ± 0.44$^c$ | 4.43 ± 0.15$^a$ | 7.06 ± 0.46$^b$ | 5.00 ± 0.33$^a$ | 5.06 ± 0.57$^a$ | 7.63 ± 0.51$^{bc}$ |
| Hb | 13.54 ± 0.56$^c$ | 6.48 ± 0.25$^a$ | 10.68 ± 0.74$^b$ | 7.64 ± 0.54$^a$ | 7.32 ± 0.91$^a$ | 11.10 ± 0.88$^b$ |
| PCV | 49.16 ± 2.47$^c$ | 25.56 ± 0.86$^a$ | 40.24 ± 2.79$^b$ | 28.66 ± 2.18$^a$ | 28.70 ± 3.34$^a$ | 41.88 ± 3.14$^{bc}$ |
| MCV | 57.16 ± 1.08$^{ab}$ | 57.70 ± 0.49$^b$ | 56.90 ± 0.44$^{ab}$ | 57.24 ± 1.13$^b$ | 56.60 ± 0.61$^{ab}$ | 54.76 ± 0.51$^a$ |
| MCH | 15.78 ± 0.27$^b$ | 14.74 ± 0.20$^a$ | 15.10 ± 0.17$^{ab}$ | 14.58 ± 0.38$^a$ | 14.36 ± 0.28$^a$ | 14.48 ± 0.20$^a$ |
| MCHC | 27.62 ± 0.45$^c$ | 25.48 ± 0.19$^{ab}$ | 26.52 ± 0.14$^b$ | 25.49 ± 0.19$^{ab}$ | 25.38 ± 0.59$^a$ | 26.48 ± 0.24$^b$ |
| PLT (×10) | 149.30 ± 9.46$^b$ | 40.80 ± 9.50$^a$ | 63.82 ± 1.29$^a$ | 47.00 ± 4.61$^a$ | 41.76 ± 5.71$^a$ | 148.94 ± 12.03$^b$ |
| LYM | 75.48 ± 4.18$^a$ | 75.60 ± 0.00$^a$ | 81.32 ± 4.89$^a$ | 75.72 ± 1.32$^a$ | 85.58 ± 2.24$^a$ | 80.90 ± 6.72$^a$ |

Each value is a mean of 5 replicates ± SEM. Columns with different superscripts were significantly different (p < 0.05); C – Control, IU – Infected untreated, IC – Infected treated with chloroquine, IL – Infected treated with lumefantrine (pure), ILC- Infected treated with lumefantrine - copper complex, NLC- Not infected but treated with lumefantrine - copper complex, WBC - White blood cell count, Hb - Haemoglobin concentration, RBC - Red blood cell count, PCV - Packed cell volume, MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin, MCHC - mean corpuscular haemoglobin concentration, PLT - Platelet count, LYM - Lymphocytes

Conversely, there was a significant increase ($p<0.05$) in the level of WBC when compared with the positive control in all the groups except the uninfected but treated with the complex group. The malaria-infected mice and treated with chloroquine and lumefantrine -copper complex groups did not show any significant difference ($p>0.05$) in the concentration of MCV when compared with the positive control; there was however a significant increase ($p<0.05$) in the malaria-infected but untreated mice.

Tables 9 and 10 show the effect of lumefantrine and lumefantrine - copper complex on chromosomal aberration in the liver and kidney of *P. berghei* NK-65 infected mice respectively. There was no significant difference ($p > 0.05$) in the total number of metaphases analyzed (TNMA) across the groups in both the liver and kidneys when compared with the positive control. Also, there was no significant difference ($p > 0.05$) between the positive control and the group administered pure lumefantrine in the amount of metaphases with chromosomal aberration (MCA); however, a significant increase ($p < 0.05$) was observed in the groups treated with lumefantrine - copper complex when compared with the control for both the liver and kidneys. The mitotic index showed no significant alteration ($p > 0.05$) across the groups in both the liver and kidneys when compared with the control. In contrast, there was a significant increase ($p < 0.05$) in the percentage chromosomal aberration across the groups except for the group treated with pure lumefantrine, that showed no significant difference ($p > 0.05$) when compared with the control.

### Discussion

Mechanochemical or solvent-less synthesis is a simple and environmentally friendly procedure (Cave *et al.*, 2001). The method gave about 76-78% yields within a shorter duration of 15 minutes as compared to 50-60% by classical solution phase which often takes 3 hours for the reaction to complete. This result agrees with the findings of Tella *et al.* (2011) who reported that infrared spectra data suggested that lumefantrine behaved as a ligand with donor sequence towards copper ions, with which coordination took place. The limitation of widespread use of solvent or even the total absence of solvent in synthetic reactions is one of the main targets of green chemistry (Mikhailanko *et al.*, 2004).

The *in vivo* antimalarial activity of lumefantrine - metal complex showed that lumefantrine - copper complex is more efficient in clearance of malaria parasite than pure lumefantrine. Lumefantrine - copper complex showed curative antimalarial activity comparable to chloroquine. Coordination of the metal improved the activity of the drug; this could be attributed to the complex binding first without being decomposed at the receptor site and also the deposition of free metal ion in the parasite membrane. These results agree with the findings of Biot *et al.* (2001) who reported that incorporation of a metal ion into a molecule gave rise to the formation of a compound with enhanced activity.

Some enzymes are present in the plasma whereas they do not originally belong in the plasma or serum. These enzymes could be present due to leakage from their original location into the plasma as a result of tissue damage. Biochemical indices that are measured in the serum, plasma and organs such as liver and kidney are useful markers in the assessment of damage to tissues. A measure of the activities of various enzymes in tissues and body fluids plays a significant function in disease diagnosis and tissue assessment (Akanji *et al.*, 1993). ALP is a marker enzyme for the integrity of the plasma membrane of hepatocytes (Akanji *et al.*, 1993) and the endoplasmic reticulum (Wright and Plumer, 1974; Shahjahan *et al.*, 2004); any alteration in the activity of the enzyme either in the tissue or serum, would be indicative of a likely damage to the outer boundary of the cells. The liver and plasma activities of the enzyme (Table 3) show a significant difference between the control and the test; this may indicate that the integrity of the plasma membrane has been impaired. This may also be attributed to the stress inflicted on the tissue by the drugs, which could have resulted in leakage of the enzyme molecule through the extracellular fluid, as was significantly noticed in the plasma. In an effort to counteract this stress, *de novo* synthesis of the enzymes might have been stimulated by the tissues, thus resulting in elevated ALP activity in the tissues as observed (Umezawa and Hooper, 1982; Malomo *et al.*, 1995).

Aspartate and alanine aminotransferases (AST and ALT) are significant marker enzymes of liver cytolysis and can be employed in assessing liver damage. The activities of AST and

Table 9. Effect of lumefantrine and lumefantrine - copper complex on chromosomal aberration in the kidney of *P. berghei* NK-65 infected mice

| Groups | TNMA | MCA | MI | %CA |
|---|---|---|---|---|
| C | $159.00 \pm 2.00^a$ | $2.00 \pm 0.30^a$ | $15.90 \pm 0.20^a$ | $1.25 \pm 0.61^a$ |
| IU | $137.00 \pm 4.00^a$ | $21.50 \pm 1.50^b$ | $13.70 \pm 0.40^a$ | $15.68 \pm 0.64^b$ |
| IC | $148.50 \pm 2.50^a$ | $39.00 \pm 6.00^c$ | $14.85 \pm 0.25^a$ | $26.09 \pm 4.74^c$ |
| IL | $149.00 \pm 2.00^a$ | $2.50 \pm 2.50^a$ | $14.90 \pm 0.20^a$ | $1.70 \pm 1.70^a$ |
| ILC | $138.50 \pm 16.50^a$ | $24.00 \pm 2.00^b$ | $13.85 \pm 1.65^a$ | $17.40 \pm 0.63^{bc}$ |
| NLC | $151.00 \pm 7.00^a$ | $39.00 \pm 3.00^c$ | $15.10 \pm 0.70^a$ | $25.98 \pm 3.19^c$ |

Each value is a mean of 5 replicates ± SEM. Columns with different superscripts were significantly different ($p < 0.05$); TNMA – Total number of metaphases analysed, MCA – Metaphases with chromosomal aberration, MI – Mitotic index, %CA – Percentage chromosomal aberration. C – Control, IU – Infected untreated, IC – Infected treated with chloroquine, IL – Infected treated with lumefantrine (pure), ILC- Infected treated with lumefantrine - copper complex, NLC- Not infected but treated with lumefantrine - copper complex

Table 10. Effect of lumefantrine and lumefantrine - copper complex on chromosomal aberration in the liver of *P. berghei* NK-65 infected mice

| Groups | TNMA | MCA | MI | %CA |
|---|---|---|---|---|
| C | $138.00 \pm 12.00^a$ | $2.00 \pm 1.00^a$ | $13.80 \pm 1.20^a$ | $1.53 \pm 0.86^a$ |
| IU | $138.00 \pm 9.00^a$ | $29.50 \pm 2.50^b$ | $13.80 \pm 0.90^a$ | $21.35 \pm 0.42^{ab}$ |
| IC | $142.50 \pm 12.00^a$ | $39.50 \pm 11.50^b$ | $14.20 \pm 1.20^a$ | $28.71 \pm 10.53^b$ |
| IL | $138.00 \pm 12.00^a$ | $3.00 \pm 2.00^a$ | $13.80 \pm 1.20^a$ | $2.32 \pm 1.65^a$ |
| ILC | $139.00 \pm 10.00^a$ | $28.00 \pm 1.00^b$ | $13.90 \pm 1.00^a$ | $20.30 \pm 2.18^{ab}$ |
| NLC | $143.00 \pm 11.00^a$ | $39.50 \pm 10.50^b$ | $14.30 \pm 1.10^a$ | $28.36 \pm 9.53^b$ |

Each value is a mean of 5 replicates ± SEM. Columns with different superscripts were significantly different ($p < 0.05$); TNMA – Total number of metaphases analysed, MCA – Metaphases with chromosomal aberration, MI – Mitotic index, %CA – Percentage chromosomal aberration. C – Control, IU – Infected untreated, IC – Infected treated with chloroquine, IL – Infected treated with lumefantrine (pure), ILC- Infected treated with lumefantrine - copper complex, NLC- Not infected but treated with lumefantrine - copper complex

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ALT in the liver and plasma (Tables 4 and 5) showed significant differences when compared with the control; these may suggest that the integrity of the plasma membrane and amino acid metabolism of these tissues have been compromised (Shahjahan *et al.*, 2004). Lactate dehydrogenase (LDH) is an enzyme that is present in almost all body tissues. It plays an important role in cellular respiration (conversion of glucose from food into usable energy for cells). Increase in the hepatic and plasma activity of the enzyme (Table 6) in both the liver and plasma when compared to the control could be as a result of induction of the enzyme possibly by *de novo* synthesis (Malomo *et al.*, 1995).

Renal function tests are often employed either to examine the presence or absence of the active laceration in the kidney, or to evaluate the proper functioning capacity of the various parts of the nephron. The main nitrogen-containing metabolic product of protein catabolism and the main catabolic product of muscle metabolism are urea and creatinine respectively; they are employed in the measurement of glomerular filtration rate. The observed difference in the plasma urea and creatinine concentrations (Table 7) when compared with the control may suggest that the drugs, except chloroquine, impaired the clearance function of the kidney.

The propagation of nerve impulses is along the nerve cells and their transmission to receptor cells are influenced by potassium ions. The sodium pump regulates the intracellular $K^+$ concentration (of 140 mM) as against the extracellular $K^+$ concentration (of 5 mM) (Horton *et al.*, 1993). The difference in concentration of $K^+$ (Table 7) observed in the test groups may indicate the interference of the drug with potassium pump, thus interrupting the maintenance of its extracellular concentration. The alteration in the concentration of sodium and bicarbonate ions in the plasma suggested that there was glomerular dysfunction (Tietz *et al.*, 1994). Chloride ion is the most abundant extracellular anion with an extracellular concentration 26 times as much as its intracellular concentration. It has the ability to permeate (by diffusion) the plasma membrane easily and its transport is closely associated with $Na^+$ movement. It plays an important role in regulating body pH levels and also in energy production. An increase in the level of chloride ion concentration in the plasma when compared to the control may be from the influx of $Na^+$ pool in body fluids.

In the tropical region of the world, malaria is a chief cause of deaths. In 2010, 219 million cases were reported globally (WHO, 2012). Some of the commonest complications of malaria include haematological changes and are deeply implicated in malaria pathogenesis. The major cell types such as RBCs, leucocytes and thrombocytes are involved in these major changes (Maina *et al.*, 2010; Bakhubaira, 2013; Warimwe *et al.*, 2013; van Wolfswinkel *et al.*, 2013). Malaria patients tended to have significantly lower platelets, WBCs, lymphocytes, eosinophils, RBCs and Hb level, while monocyte and neutrophil counts are often significantly higher in comparison to non-infected patients (Gerardin *et al.*, 2002; Erhart *et al.*, 2004; Adedapo *et al.*, 2007).

Decrease in haemoglobin levels might be accompanied by a fall in the RBC and PCV (Moss, 1999). The main role of RBC is haemoglobin transport, which then transfers oxygen from the lungs to the tissues (Waugh and Grant, 2001). The decrease in red cell might be due to the decrease in the production of erythrocytes. It may also show that the drug lacked erythropoietic potentials. Very low levels of red blood cells, haemoglobin and haematocrits can predispose to anaemia (Muhammad and Oloyede, 2009). This might be that the oxygen carrying capacity of the blood and the amount of oxygen delivered to the tissues were reduced by the drug administered. The significant increase in white cells might be an indication that there was a challenge on the immune systems. Increased level of neutrophils may also indicate inflammatory response due to the toxic effect of the drug on the tissues. Neutrophils are mobilised to the location where an injury has occurred within minutes and are the hallmark of acute inflammations (Cohen *et al.*, 2002). Blood indices such as mean corpuscular haemoglobin concentration (MCHC) are of significance in the diagnosis of anaemia in most animals. The significant decrease observed in haemoglobin, PCV, MCH and PLT (Table 8) may suggest that administration of lumefantrine - copper complex have a pathological effect on the blood parameters. Increase in the levels of WBC and LYM when compared to the control may be suggestive of defence mechanism against malaria parasite and/ or lumefantrine - copper complex.

The significant increase in the frequencies of chromosomal aberrations in the mice treated with lumefantrine - copper complex when compared with pure lumefantrine may be that lumefantrine - copper complex is more efficient in the treatment of malaria and may be more likely to increase DNA damage if used over a long period of time. This observation is consistent with the findings reported by Yadav and Trivedi (2009) in which the exposure of *Channa punctatus* to mercuric chloride, arsenic trioxide and copper sulphate pentahydrate for a week caused chromatid and chromosome breaks, chromatid and chromosome gaps, along with the ring and di-centric chromosomes in the kidney cells. The findings indicate the genotoxic potential of these metals, even in sub-lethal concentrations (Rakshit and Gangopadhyay, 2015). Similar results have also been reported in Swiss albino mice after oral, subcutaneous and intraperitoneal exposures to copper sulphate. Fahmy (2000) reported teratogenic effects of copper in carp at much lower copper concentrations (0.051 mg/L at pH 7.6 and 0.019 mg/L at pH 6.3) where deformed head, spinal column, upper jaw and a small or absent swim bladder was reported. Copper toxicity is believed to be due to nonspecific binding of the reactive metal cation ($Cu^{2+}$) to biologically important molecules (Rakshit and Gangopadhyay, 2015).

**Conclusions**

Mechanochemical synthesis of lumefantrine - copper complex is an environmentally friendly process. The antimalarial activity of the complex investigated using mice infected with *P. berghei* NK-65 showed that the complex exhibited higher anti-malarial activity in comparison with pure lumefantrine and also compared favourably with the standard drug (chloroquine). The biochemical parameters investigated in this study suggested functional and selective toxicity and some chromosomal damage caused by the pure and complex drug; therefore, lumefantrine - copper complex may not be completely safe for repeated oral treatment of malaria.

## Acknowledgements

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