Original Research Article

Journal of Drug Discovery and Research (*JDDR*) June 2022; 1(1): 16-24
Journal of Drug Discovery Research Group, Faculty of Pharmacy, University of Uyo, Nigeria
Available online at http://www.ddrg.net

Effect of ritonavir/lopinavir on the efficacy of chloroquine and artemether/lumefantrine in mice

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Reviewed: 09/06/2022 *Accepted: 21/06/2022*

ABSTRACT

***Background*:** Malaria co-infection with Human Immunodeficiency Virus (HIV) is common and its pharmacotherapy combines antiretroviral with antimalarial drugs, hence drug-drug interaction is inevitable. This study reports the effect of an antiretroviral drug, lopinavir/ritonavir (LPV/r) on the antimalarial activity of artemether/lumefantrine (AL) and chloroquine (CQ) in a mouse model of *Plasmodium berghei.*

Methodology: The standard procedures of prophylactic, suppressive and curative antiplasmodial assay models were adopted. The mice were divided into 6 groups of 5 mice each and 10 mg/kg, 6 mg/kg and 5 mg/kg body weight of CQ, LPV/r and AL were administered to groups 1, 2 and 3, respectively. The same dose of LPV/r and CQ was administered to group 4 concurrently, while group 5 received a similar dose of LPV/r and AL, concurrently. The mice in group 6 served as negative control.

Result: The study revealed that the co-administration of LPV/r with CQ and AL did not affect the suppressive antiplasmodial effect of CQ but boosted the parasite clearance of AL by 17.78 %. In the prophylactic test, the co-administration of LPV/r with CQ and AL also boosted the parasite clearance of CQ by 18.04 % and slightly boosted the parasite clearance of AL by 3.14 %. However, there was no significant effect of LPV/r on CQ and AL in the curative study.

Conclusion: Therefore, concurrent administration of LPV/r with AL and CQ affects mostly the suppressive and prophylactic effectiveness of AL and CQ, respectively.

Keywords : Artemether-lumefantrine, chloroquine, drug-drug interaction, lopinavir-ritonavir, *Plasmodium berghei.*

1. INTRODUCTION

Malaria and HIV, either as an individual ailment or in co-infection, remain the leading cause of global mortality to date[1]. The distribution of HIV and malaria is similar across the world and the severity of one seems to be enhanced by the presence of the other in cases of co-infection. Malaria-endemic areas, especially in the sub-Saharan region, witness higher rates of malaria transmission among HIV patients[2]. Malaria infection is known to stimulate radical immune mechanisms that usually result in the activation of HIV replication that is capable of causing a transient increase in HIV viral load[3],[4],[5]. Malaria and HIV co-infection are found to be associated with increased frequency of clinical parasitaemia and severe malaria; increased parasite and viral load; and impaired immunity to malaria in non-pregnant adults, children and pregnant women[6]. There is also impaired antimalarial drug efficacy in non-pregnant adults and pregnant women concurrently receiving antimalarials and antiretrovirals[7]. All these lead to an

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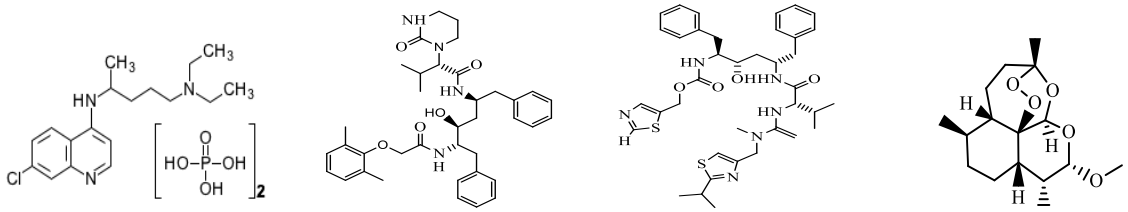
increased rate of malaria treatment failure and various reports suspect treatment failure to occur due to re-infection with new malaria strains rather than a recrudescence of prior infection[8],[9],[10],[6]. The combination of malaria and HIV attack has resulted in more than 2 million deaths yearly because of the massive geographical overlap between the two diseases. In 2020, about 241 million cases and 627,000 deaths occurred due to malaria infection globally. A critical analysis of the report reveals that about 95 % of the burden and 96 % of deaths occurred in sub-Saharan Africa with about 80 % of the deaths occurring among children less than 5 years. Nigeria accounts for the highest proportion of the global and regional malaria death of 31.9 % among the four African countries that together account for more than half of the deaths globally [11]. Again, the 2020 global HIV and AIDs statistical report [12] reveal that an estimated 37.7 million people were living with HIV among which 1.7 million were children and 6.1 million of these people are ignorant of their HIV status; hence new infections keep surging daily[13]. The 2020 statistical analysis shows that 680,000 deaths occurred in the year under consideration as a result of AIDs-related sickness. The African region is always the worst affected and in every 25 adults, 1 is living with HIV. Comparatively, the African region accounts for more than two-thirds of the global HIV cases. The treatment of HIV is often complicated because it is a host-specific infection whose pathogenesis is complex and varies among patients. Effective management of HIV infection is done using antiretroviral therapy (ART). Standard ART comprises at least three medicines, called highly active antiretroviral therapy (HAART). This effectively controls the multiplication of HIV in infected people, slowing the disease progression and reducing the risk for infection [14]. Lopinavir/ritonavir, a protease inhibitor, was approved for use in the year 2000 by the United States Food and Drug Administration. It is effective in both antiretroviral naive and experienced HIV-infected patients around the world [15]. The preferred drugs for the treatment of malaria over the years the world over have been quinine, mepacrine, chloroquine, mefloquine and halofantrine. Nevertheless, these drugs have been replaced globally by artemisinin and its derivatives in the treatment of malaria now [16]. Antiretroviral drugs, specifically the non-nucleoside reverse transcriptase inhibitors and protease inhibitors, are potent inducers and/or inhibitors of cytochrome (CYP) enzymes and transporter proteins, with potential for drug–drug interactions when co-administered with other drugs [5,17]. Significant interactions between chloroquine and protease inhibitors like ritonavir at prophylactic dosing drug concentrations have been reported [8]. Studies have also revealed significant changes in the plasma drug concentration in healthy volunteers given lumefantrine with lopinavir-ritonavir [5]. Lopinavir and ritonavir are inhibitors of the intestinal and hepatic activity of CYP3A4, although lopinavir is a less potent inhibitor of the CYP34 than ritonavir. Ritonavir is also known to inhibit CYP2C9 [18,19]. So co-administration with artemether/lumefantrine or chloroquine may result in increased chloroquine, artemether and lumefantrine plasma concentrations, with attendant unwanted consequences. Lopinavir and ritonavir are also known to induce the hepatic activity of cytochrome P450 enzymes including CYP2C9, CYP2C19, and CYP1A2 [20]. Ritonavir is metabolized by CYP3A4 and it is also an inhibitor of the enzyme [21]. The metabolism of artemether and lumefantrine is predominantly mediated by CYP3A4/5. To a lesser extent, artemether and lumefantrine are also metabolized by CYP2B6, CYP2C9, CYP2C19 and possibly by CYP2A6 [22,23,24,25]. Chloroquine and hydroxychloroquine are also metabolized predominantly by CYP2C8 and 3A4/5 *in vivo* by N-deethylation, although chloroquine is effectively inhibited by CYP2D6 *in vivo* majorly in individuals with limited CYP2D6 activity [20, 26,27,28]. CYP3A4 is significant in the metabolism of chloroquine, artemether/lumefantrine and lopinavir/ritonavir combination. Therefore, this study seeks to determine the effect of concomitant administration of Lopinavir-ritonavir on the efficacy of chloroquine and Artemether/Lumefantrine in mice.



(a) (b) (c) (d)

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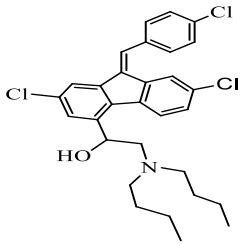

(e)

Figure 1: Chemical structure of (a) chloroquine phosphate (b) lopinavir (c) ritonavir (d) artemether (e) lumefantrine

2. MATERIALS AND METHODS

2.1 Materials

Lopinavir/ritonavir (Aluvia™) (AbbVieDeutschland GmbH & Co. KG, Knollstasse, Ludwigshafen, Germany), artemether/lumefantrine (Emzor Pharmaceuticals, Nigeria) and chloroquine phosphate (May & Baker Pharmaceuticals, Nigeria) used in this study were sourced from the Central Pharmacy store of University of Uyo Teaching Hospital, Nigeria. They were kind gift from the Head of Department of the Pharmacy Unit. The chloroform (Surechem LTD, United Kingdom) used to anaesthetise the animals was supplied by Bristol Scientific, Nigeria and the sterile normal saline (JUHEL Nigeria Limited) used to prepare the final inoculum was purchased from a Pharmacy shop in Uyo metropolis. The Chloroquine sensitive strain of *Plasmodium berghei* (ANKA) was obtained from the National Institute of Medical Research (NIMER) in Lagos, Nigeria and maintained by sub-passage in Albino mice. Both male and female mice weighing between 15 and 25 grams were used in the study. They were obtained from the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria. The protocol for this work was approved by the Faculty of Pharmacy Ethical Approval Institutional Animal Care and Use Committee, University of Uyo, Nigeria.

2.2 Methods

2.2.1 Animal Stocking and grouping

A total of 90 Albino mice were separated into male and female and held in separate cages. They were fed with grower pellet feed and water was given *ad libitum*. The mice were allowed to stay together for 7 days to acclimatize before being used for the study. The mice were randomly divided into 3 groups (1, 2 and 3) of 30 mice each corresponding to the suppressive, prophylactic and curative models. In each group, the mice were further sub-divided into 6 sub-groups of 5 mice each and their weights were recorded.

2.2.2 Preparation of stock solution of drugs

The methods adopted in the preparation of the stock solutions were all modified and in all cases, the working solutions were properly stirred before administration.

Chloroquine phosphate: Twenty tablets were crushed and 50 mg of the powder was accurately weighed and transfered into a 100 mL volumetric flask. About 40 mL of distilled water was added and the flask was sonicated for 20 minutes. The flask was filled to mark with more distilled water to give a stock solution of 500 µg/mL [29].

Artemether lumefantrine: A portion of twenty tablets finely powdered, equivalent to 10 mg artemether and 60 mg lumefantrine, was transferred into a 100 mL volumetric flask and 60 mL of distilled water was added. The solution was sonicated for 20 minutes with occational shaking and diluted to volume with distlled water to give a stock solution of 0.1 mg/mL and 0.6 mg/mL of artemether and lumefantrine, respectively [30].

Lopinavir/ritonavir: Twenty tablets, each containing 200 mg of lopinavir and 50 mg of ritonavir were crush into powder and 50 mg of powder was weighed and dissolved in 100 mL of distilled water. The solution was sonicated for 20 min with occasional shaking to give a stock solution of 500 µg/mL of lopinavir and 125 µg/mL of ritonavir concentration [31].

2.2.3 Parasite Inoculation and drug administration

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An albino mouse earlier infected with *Plasmodium berghei* (ANKA strain) was used as a donor. The parasite load was ascertained through actual parasite count from a thin smear made from blood gotten from the tail vein of the mouse. The mouse was anaesthetised with chloroform in an enclosure and blood was obtained through the cardiac puncture into a sterile heparinized bottle. The volume of blood obtained from the donor mouse was diluted with sterile normal saline so that the final inoculum consisted of 5×10^7/mL of *Plasmodium berghei* infested parasitized erythrocytes. Each mouse was inoculated through the intraperitoneal route with 0.2 mL of infected blood which contained 1.0×10^7 parasitized red blood cells, the standard inoculum for the infection of a single mouse[32]. A dose of 10 mg/kg body weight of chloroquine phosphate (CQ, 250 mg) was administered to all animals that received the drug, 6 mg/kg body weight of lopinavir/ritonavir (LPV/r, 200 mg/50 mg) was administered to those in groups that received the drug, while 5 mg/kg body weight of artemether/lumefantrine (AL, 80 mg/480 mg) was administered to those in groups that received the drug. All administrations were done by oral gavage using a cannula.

2.2.4 Antimalarial tests

The antimalarial models of Knight and Peters (1980), Peters (1965) and Ryley and Peters (1970) used for suppressive, prophylactic and curative models, respectively as described by Okokon *et al.*[32] were adopted with slight modifications in the antimalarial study.

Determination of Suppressive Activity (4-day test): This test was used to evaluate the schizonticidal activity of the drugs and their combinations against early *Plasmodium berghei* infection in mice. Grouping was done as discussed earlier and group 1 was used in this model of analysis. On the first day (D_0), the 30 mice were infected with the parasite. The mice in sub-groups 1, 2 and 3 were administered with 10 mg/kg CQ (positive control), 6 mg/kg LPV/r, and 5 mg/kg AL, respectively. Those in sub-groups 4, 5 and 6 received 6 mg/kg LPV/r + 10 mg/kg CQ, 6 mg/kg LPV/r + 5 mg/kg AL and distilled water (negative control), respectively. The parasitaemia level was determined by counting the number of parasitized erythrocytes out of 100 erythrocytes randomly in 8 fields of the microscope.

Parasitaemia was determined by the formula[33]:

$$\%\ Parasitaemia\ = \frac{Numberofparasitized\ RBC}{Number\ of\ RBC\ Counted} \times 100$$

The average percentage chemo-suppression was calculated using the formula: $100\ \left(\frac{A-B}{A}\right)$

Where A= Average percentage parasitaemia in the negative control.
B = Average percentage parasitaemia in the test group.

Determination of Repository/prophylactic activity: The mice in group 2 were used for this model and the sub-grouping and drug administration were similar to the suppressive test. All the mice in the sub-groups were treated for 3 consecutive days ($D_0 - D_2$) and on day 4 (D_3), they were intraperitoneally injected with 0.2 mL of the infected blood. The parasite density was assessed using thin films obtained from the tail blood of each mouse after 72 hours of inoculation.

Determination of Effect of LPV/r, AL and CQ on established infection (Curative or Rane's test): The mice in group 3 were inoculated intraperitoneally with standard inoculums of 1×10^7/mL of *Plasmodium berghei* parasitized red blood cells on the first day (D_0). Exactly 72 hours later (D_3), blood smears from tail snip of the animals were obtained for parasite count to determine baseline infection levels. The randomized sub-grouping and administration of drugs were done as in suppressive model described above. All the drugs were administered once a day for 5 days. Tail blood smears were obtained from the animals at regular intervals during the treatment period (D_5, D_7, D_9). These were used to prepare thin films and used to monitor the level of parasitaemia. The Mean Survival Time (MST) of each group was determined over 28 days ($D_0 - D_{27}$) using the formula[34]:

$$MST = \frac{Sum\ of\ Surfival\ time\ (days) of\ all\ mice\ in\ group}{Total\ number\ of\ mice\ in\ that\ group}$$

2.3 Statistical analysis

The statistical analysis was performed using GraphPad Prism version 5.0 for Windows. A two-way ANOVA was used to ascertain the effect of lopinavir/ritonavir on the efficacy of chloroquine and artemether/lumefantrine on concurrent administration with antimalarial drugs. Statistical significance was considered at $p < 0.05$.

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3. RESULTS

The results of the findings were averaged, the standard error of mean (SEM) was calculated and tabulated according to each model of the study. The results are presented in bar charts with error bars representing SEM in each group.

3.1 Four Day Suppressive Effect

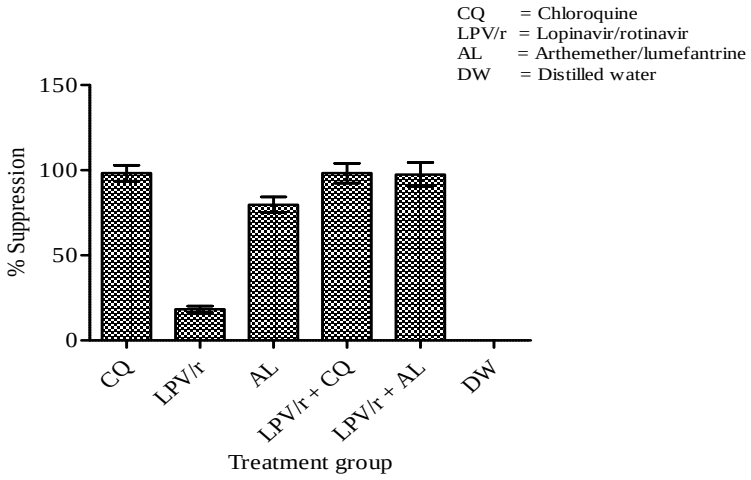

Figure 2: Effect of LPV/r on the suppressive activity of CQ and AL in *P. berghei*-infected mice

All treatment groups showed statistically significant ($p < 0.05$) chemosuppressive activity against *P. berghei* infection in mice relative to the negative control (Figure 2). The highest level of inhibition (98.25 %) was shown by the positive control group treated with chloroquine (CQ), followed by the group treated with lopinavir/ritonavir and artemether-lumefantrine (LPV/r + AL) (97.49 %), then the group treated with artemether/lumefantrine (AL) (79.71 %) and the least inhibition power (18.31 %) was displayed by the group treated with lopinavir/ritonavir (LPV/r). However, there was no statistically significant ($p < 0.05$) chemosuppression between the groups treated with CQ only (98.25 %) and LPV/r + CQ (98.25 %). Also the groups treated with AL only (79.71 %) and LPV/r + AL (97.49 %) did not show any statistical significance.

3.2 Prophylactic Test

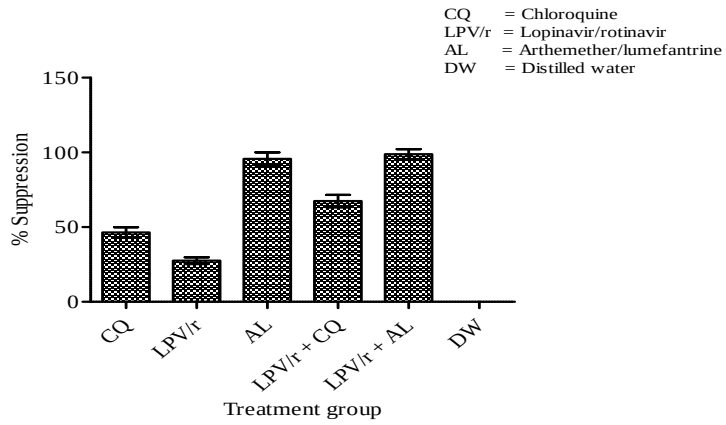

Figure 3: Effect of LPV/r on the prophylactic activity of CQ and AL in *P. berghei*-infected mice

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Just like in the suppressive test, all treatment groups in the repository test showed statistically significant ($p < 0.05$) chemosuppressive activity against *P. berghei* infection in mice relative to the negative control (Figure 3). The highest level of inhibition (98.84 %) was shown by the group treated with lopinavir/ritonavir and artemether-lumefantrine (LPV/r + AL), followed by the group treated with artemether-lumefantrine (AL) only (95.70 %). The group that received lopinavir/ritonavir and chloroquine (LPV/r + CQ) recorded a 64.44 % inhibition level while the group administered chloroquine (CQ) only and lopinavir/ritonavir (LPV/r) only recorded 46.40 % and 27.56 % inhibition respectively. However, the groups treated with LPV/r + AL and LPV/r + CQ combination did not show any statistical significant ($p < 0.05$) difference from the groups given AL only and CQ only, respectively.

3.3 Curative Test

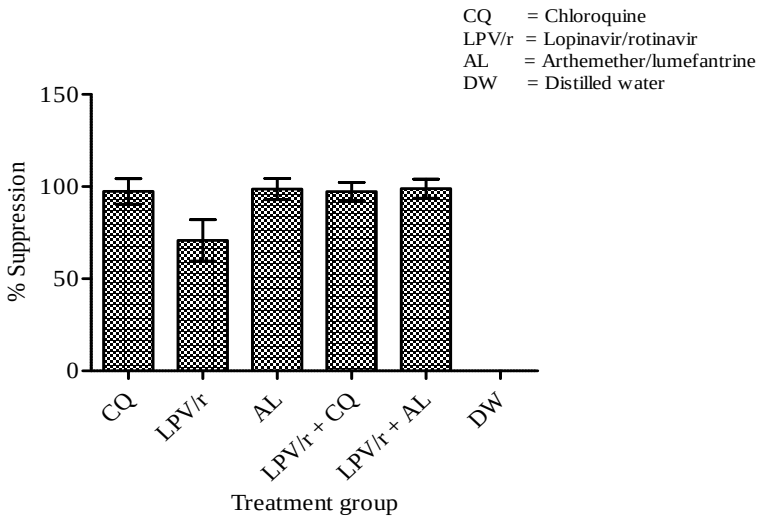

Figure 4: Effect of LPV/r on the curative activity of CQ and AL in *P. berghei*-infected mice after 5 days of treatment

The curative result after 5 days of treatment (Figure 4) showed a competitive result in all treatment groups except the group treated with LPV/r. The percentage parasitaemia reduction among the treatment groups showed a progression from group treated with LPV/r only (70.85 %) to LPV/r + CQ (97.29 %), to CQ only (97.39 %), to AL only (98.69 %) and to LPV/r + AL (98.89 %). Just like in other two models, there was no statistical significance ($p < 0.05$) in the study groups (CQ only vs LPV/r + CQ and AL only vs LPV/r + AL). The table below shows the mean survival time (MST) of mice in the various treatment groups at the end of the study.

Table 1: The Mean Survival Time (MST: days) of mice receiving LPV/r, AL, and CQ alone and in combination during established infection

Drug	Dose in mg/kg/day	Mean survival time (MST) (days)
DW	10 mL	10.60
LPV/r	6	19.80
CQ	10	28.00^{+}
AL	5	27.60
LPV/r + CQ	6 + 10	28.00^{+}
LPV/r + AL	6 + 5	28.00^{+}

LPV/r = lopinavir/ritonavir, CQ = Chloroquine, AL = Artemether/lumefantrine, DW = Distilled water

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4. DISCUSSION

This study evaluated the effect of co-administration of lopinavir/ritonavir (LPV/r) on the antimalarial effect of chloroquine (CQ) and artemether/lumefantrine (AL) in a mouse model of *P. berghei.* The *in vivo* method was used to evaluate the antiplasmodial effects of the drugs because it allows the possible prodrug effect and likely boosting of the immune system in the eradication of the infectious agents. Moreover, rodent models have been validated over the years through the discovery of several antimalarials like chloroquine and artemisinin derivatives [35]. In the four-day parasitaemia suppression study, treatment with LPV/r (6 mg/kg) resulted in a very low parasite suppression compared to the positive control (CQ only). Parasitaemia in the untreated control and LPV/r alone treated animals were 0 and 18.31 % respectively. This was lower than values reported by Abiodun *et al.* [36] of 2.5 to 37.4% and 1.3 to 12.2%, respectively, on day 3–9 post-infection. However, the study of Abiodun *et al*. [36], shows the low parasite suppression capability of LPV/r alone and it confirms that the treatment with LPV/r alone does not appear to be effective in suppressive treatment with infection of *P. berghei* in mice. However, the study revealed that the co-administration of LPV/r with CQ did not affect the suppressive antiplasmodial effect of CQ. The parasite clearance of the CQ + LPV/r treated group did not differ from the parasite clearance of the group treated with CQ alone. The same effect was observed in the combination of LPV/r with AL. The parasite clearance of AL was statistically ($p < 0.05$) unaffected by the co-administration of LPV/r. This is similar to the observation of Abiodun *et al.* [37], who reported that the treatment with AL alone or combined with LPV/r caused a complete parasite suppression. However, the co-administration of LPV/r with AL boosted the parasite clearance of AL from 79.71 % to 97.49 %. The Prophylactic activity of the combination of LPV/r with CQ and AL respectively was determined. The parasite suppressive activity of CQ alone and AL alone was low. However, it appears that LPV/r increases the prophylactic activity of CQ. On the contrary, LPV/r did not affect the chemosuppression of AL. The chemosuppression level in groups treated with AL (95.70 ± 4.33) and those treated with LPV/r + AL (98.84 ± 3.33) showed non-significant prophylactic activity. This result is consistent with the report of Abiodun *et al.* [36,37]. They opined that LPV/r + AL resulted in dose-dependent parasite suppression. It is on record that both artemether and lumefantrine are metabolized predominantly by CYP3A4. lopinavir and ritonavir are inhibitors of CYP3A4, so co-administration with artemether/lumefantrine may result in increased artemether and lumefantrine plasma concentrations leading to higher activity [5, 37, 38]. In the curative test, LPV/r alone did not achieve complete parasite clearance and neither did it ensure the survival of the animals. However, the antimalarial drugs, CQ and AL achieved maximum parasite clearance on day 5, with 97.39 % and 98.69 % clearance, respectively and the animals in those groups survived beyond 28 days (Table 1). In the groups where LPV/r was combined with CQ and AL, however, a comparable daily reduction in parasitaemia was observed in the groups treated with CQ alone and AL alone. Moreover, the combination of LPV/r with CQ and AL had a comparable mean survival time (MST) to the single treatment with ACT and CQ, respectively. This was, however, contradictory to the findings of Abiodun *et al.*[36], who reported that AL alone did not possess a curative effect on plasmodial infection, whereas, the combination of AL and LPV/r ensured the complete suppression of parasites without recrudescence. In that report, the combination of LPV/r with AL did not produce MST similar to that of AL alone.

5. CONCLUSION

This study has demonstrated that co-administration of LPV/r with CQ and AL does not significantly affect the antimalarial effects of these drugs. The survival time of the animals treated with the combination was not also significantly altered.

Acknowledgements
We thank Mr Nsikan Malachy in the department of Pharmacology and Toxicology for his technical support during the study.

Conflict of Interest
There are no conflicts of interest.

Contribution of the Authors
A. S. E. designed and developed the research study and analyzed and discussed data; V. U. A. and A. O. N. provided materials, E. E. A. and A. O. N. performed experiments, and wrote the paper; A. S. E., A. E U. and N. A. O. reviewed and discussed the experimental data, and wrote the final manuscript. All authors read and approved the final manuscript.

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