

## Brief Research Report

# Repeated Treatment with Antimalarial Agents Causes *In Vitro* Liver Toxicity

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orcid. 0000-0002-9188-9620; E-mail: [audud@funaab.edu.ng](mailto:audud@funaab.edu.ng)**Article information****Received:** November 14<sup>th</sup>, 2022; **Revised:** December 22<sup>nd</sup>, 2022; **Accepted:** January 3<sup>rd</sup>, 2023; **Published:** January 10<sup>th</sup>, 2023**Cite this article**Audu D, Patel VB, Petagine L, Idowu OA, Idowu AB. Repeated treatment with antimalarial agents causes *in vitro* liver toxicity. *Liver Res Open J.* 2023; 4(1): 1-6.doi: [10.17140/LROJ-4-113](https://doi.org/10.17140/LROJ-4-113)**ABSTRACT****Aim**

In Africa's malaria-endemic regions, artesunate/amodiaquine (A/A) and artemether/lumefantrine (A/L) are the antimalarial medications that are most frequently prescribed. Antimalarial medications could be overused if they are self-prescribed based on symptoms rather than a parasitological diagnosis. This investigation looked at potential cytotoxic and oxidative stress effects following three dosage treatments spaced 24 h apart.

**Method**

Artesunate (50  $\mu$ M and 100  $\mu$ M), Amodiaquine (1  $\mu$ M and 10  $\mu$ M), Artemether (200  $\mu$ M and 400  $\mu$ M), and Lumefantrine (200  $\mu$ M and 400  $\mu$ M) were administered to HepG2-derived VL-17A for up to 72 h.

**Result**

With a downward trend from 24 h to 48 h to 72 h, the study findings showed that repeated administration of these medications greatly reduced cell viability. Additionally, for artemether 200  $\mu$ M treatment, the reactive oxygen species (ROS) levels increased after 24 h and 48 h but considerably decreased after 72 h. The ROS levels in artesunate, amodiaquine, artemether 400  $\mu$ M, and lumefantrine were also noticeably lower after 72 h.

**Conclusion**

The results of this experiment show that repeated applications of anti-malaria drugs, A/L and A/A to HepG2 liver cells reduced their viability in a manner that was consistent. These findings have significant implications for those who use antimalarial medications as a preventative measure without a diagnosis of parasite infection.

**Keywords**

Cell death; Reactive oxygen species; Artesunate; Amodiaquine; Artemether; Lumefantrine; Malaria.

**INTRODUCTION**

The parasite *Plasmodium* causes the disease known as malaria, which is transmitted through the bite of an infected female anopheles mosquito.<sup>1</sup> In 84 countries where malaria is endemic, there were 247 million cases worldwide in 2021, up from 245 million in 2020. African nations accounted for most of the increase.<sup>2</sup> Indoor residual spraying, long-lasting insecticidal nets, prophylactic medications, and untreated nets are some methods of preventing malaria.<sup>1,3</sup> Antimalarial medications are the only effective treatment recommended by the World Health Organization (WHO) following a malaria infection.<sup>1,4</sup>

Artesunate/amodiaquine (A/A) and artemether/lumefantrine (A/L) are the most frequently prescribed artemisinin-based combination therapy (ACT) in endemic African nations. With a cure rate of more than 90%, these medications are thought to be particularly effective against the malaria parasite.<sup>5</sup> Most ACT partner medications, including lumefantrine and amodiaquine, function similarly by the development of reactive oxygen species (ROS),<sup>6</sup> which is hypothesised to disrupt the parasite life cycle.<sup>7</sup> However, ROS can also damage lipids, proteins, and nucleic acids, causing cellular injury.<sup>8</sup> Repeated use of antimalarial drugs in the absence of infection is majorly due to (i) preventative treatment, (ii) self-prescription in the absence of parasitological confirma-

tion of malaria, and (iii) free access to these drugs in pharmacies.<sup>9</sup> The liver, central nervous system, cardiovascular system, gastrointestinal tract, and vision may all be negatively impacted by this regular use of antimalaria medications.<sup>1</sup> One component of the risk-benefit analysis is based on a medical justification for using the drug to treat or prevent malaria, despite possible antimalarial toxicity.<sup>10</sup>

The possible repeated use of the components drug of A/A and A/L has not been adequately investigated for cellular toxicity. Therefore, evaluating the potential adverse effects of this drug administration is crucial since adverse drug effects have been connected to carcinogenesis, liver damage, bone marrow toxicity, and developmental abnormalities in foetuses.<sup>11</sup> Furthermore, the repeated use of antimalarial may cause liver toxicity.<sup>11</sup> There is also a possibility that the drug's destructive effect on parasites may also contribute to its toxicity in healthy cells.<sup>12</sup> Therefore, this study investigated the cytotoxic effects of repeated exposure of artesunate, amodiaquine, artemether, and lumefantrine in hepatic HepG2 cells.

## METHODS

### Cell Culture

HepG2 (VL-17A), human hepatoma cells were maintained at 37 °C in 5% CO<sub>2</sub> in a high-glucose (25 mmol) Dulbecco's Modified Eagle Medium (DMEM) supplemented with L-Glutamine (2 mmol), penicillin (100 U/mL), streptomycin (100 mg/mL) (Lonza Ltd, United Kingdom), and supplemented with sodium pyruvate (1 mmol) and 10% foetal calf serum (Biosera, United Kingdom). Prior to maintenance, cells were cultured for four weeks in plasmocin prophylactic (5 mg/mL in DMEM).<sup>13</sup>

### Treatments

Dose response experiments were undertaken to choose a concentration that resulted in approximately 50% loss in cell viability. In our initial studies, cells were exposed to artesunate, amodiaquine, artemether, and lumefantrine at concentrations of 1 µM, 10 µM, 25 µM, 50 µM, 100 µM, 200 µM, 400 µM and 600 µM. Based on this information, the final drug concentrations used were artesunate (50 µM and 100 µM), amodiaquine (1 µM, 10 µM), artemether and lumefantrine (200 µM, 400 µM). Each concentration was applied at time 0 for the 24 h time point and fresh drug was administered after 24 h for the 48 h time point, and again at 48 h for the 72 h time point. Antimalarial were purchased from Sigma-Aldrich (Gillingham, United Kingdom). Following treatment, cell viability and ROS levels were studied. Experimental controls were treated with 0.5% dimethylsulfoxide (DMSO) in maintenance medium. Comparisons were made between the treated and untreated control groups.<sup>13</sup> This research complies to the local institution board.

### Cell Viability Assay

Cell viability was assessed using the MTT assay. HepG2 cells (25,000 cells/200 µL DMEM/well) were incubated overnight in

96-well plates and then treated with the different concentrations of drugs for 24, 48 and 72 h. After the treatment, MTT (5 mg/mL) (Sigma-Aldrich, United Kingdom) was added to each well and the cells were incubated for 2 h at 37 °C. Ninety-six (96)-well plates were removed from the incubator, and media was discarded from the wells. One hundred (100) µL DMSO was added to each well and left for 15 min at room temperature. The absorbance was read at 550 nm using a VersaMax microplate reader (Molecular Devices, UK).<sup>13</sup>

### Measurement of Intracellular Reactive Oxygen Species Levels

DCF-DA assay was used to measure cellular ROS production.<sup>14</sup> HepG2 cells (10000 cells/ 200 µL DMEM) were incubated overnight in a 96-well plate. Following antimalaria drug treatment for 24 h, 48 h and 72 h, the cells were treated with 1 µmol 2',7'-dichlorofluorescein diacetate (Sigma-Aldrich, United Kingdom) in phosphate-buffered saline (PBS) and incubated for 45 min at 37 °C. fluorescence intensity was measured at an excitation of 485 nm and an emission of 535 nm using a FLUOstar® OPTIMA (Jencons-PLS, UK). Fluorescence levels were expressed as percentage of the control.<sup>13</sup>

### Statistical Analysis

GraphPad Prism 9.0 was used for data analysis. A one-way analysis of variance (ANOVA) was conducted, followed by a Tukey post-hoc analysis, to ascertain the significant difference between the treatment groups and the control. Significant difference from the control group was determined at  $p < 0.05$ .

## RESULTS

### Repeated Treatment with Antimalaria Drug Induce Cytotoxicity

Exposure to the antimalaria drug significantly reduces cell viability, in a decreasing trend after 24 h, 48 h and 72 h compared to the control group (Figures 1 A, B and C). Treatment of cells with Artesunate 50 µM reduced cell viability by 32% ( $p < 0.01$ ), 72% ( $p < 0.001$ ), and 97% ( $p < 0.001$ ) for 24 h, 48 h and 72 h, respectively compared to the control; Treatment of cells with Artesunate 100 µM reduced cell viability by 58% ( $p < 0.001$ ), 97% ( $p < 0.001$ ) and 101% ( $p < 0.001$ ) for 24 h, 48 h and 72 h respectively compared to the control (Figures 1 A, B and C).

Exposure of cells to amodiaquine 1 µM reduced cell viability by 4%, 18%, ( $p < 0.05$ ), 21% ( $p < 0.001$ ) for 24 h, 48 h and 72 h respectively, compared to the control group; while treatment of the cell with Amodiaquine 10 µM, reduced cell viability by 34% ( $p < 0.001$ ), 89% ( $p < 0.001$ ), 94% ( $p < 0.001$ ) for 24 h, 48 h and 72 h, respectively compared to the control group (Figures 1 A, B and C).

Cells treatment with Artemether 200 µM reduced cell viability by 21% ( $p < 0.05$ ), 39% ( $p < 0.001$ ) and 59% ( $p < 0.001$ ) for 24 h, 48 h and 72 h respectively compared to the control group; while cells exposure to Artemether 400 µM reduced cell viability by 31% ( $p < 0.01$ ), 58% ( $p < 0.001$ ), 82% ( $p < 0.001$ ) for 24 h, 48 h and 72 h, respectively compared to the control group (Figures 1

A, B and C).

Treatment of cells with Lumefantrine 200  $\mu\text{M}$  reduced cell viability by 74% ( $p < 0.001$ ), 91% ( $p < 0.001$ ), 94% ( $p < 0.001$ ) for 24 h, 48 h and 72 h respectively compared to the control group; While exposure of cells with Lumefantrine 400  $\mu\text{M}$  reduced cell viability by 85% ( $p < 0.001$ ), 96% ( $p < 0.001$ ), 95% ( $p < 0.001$ ) for 24 h, 48 h and 72 h, respectively compared to the control group (Figures 1 A, B and C).

**Effects of Repeated Treatment with Antimalaria Drug on Intracellular Reactive Oxygen Species**

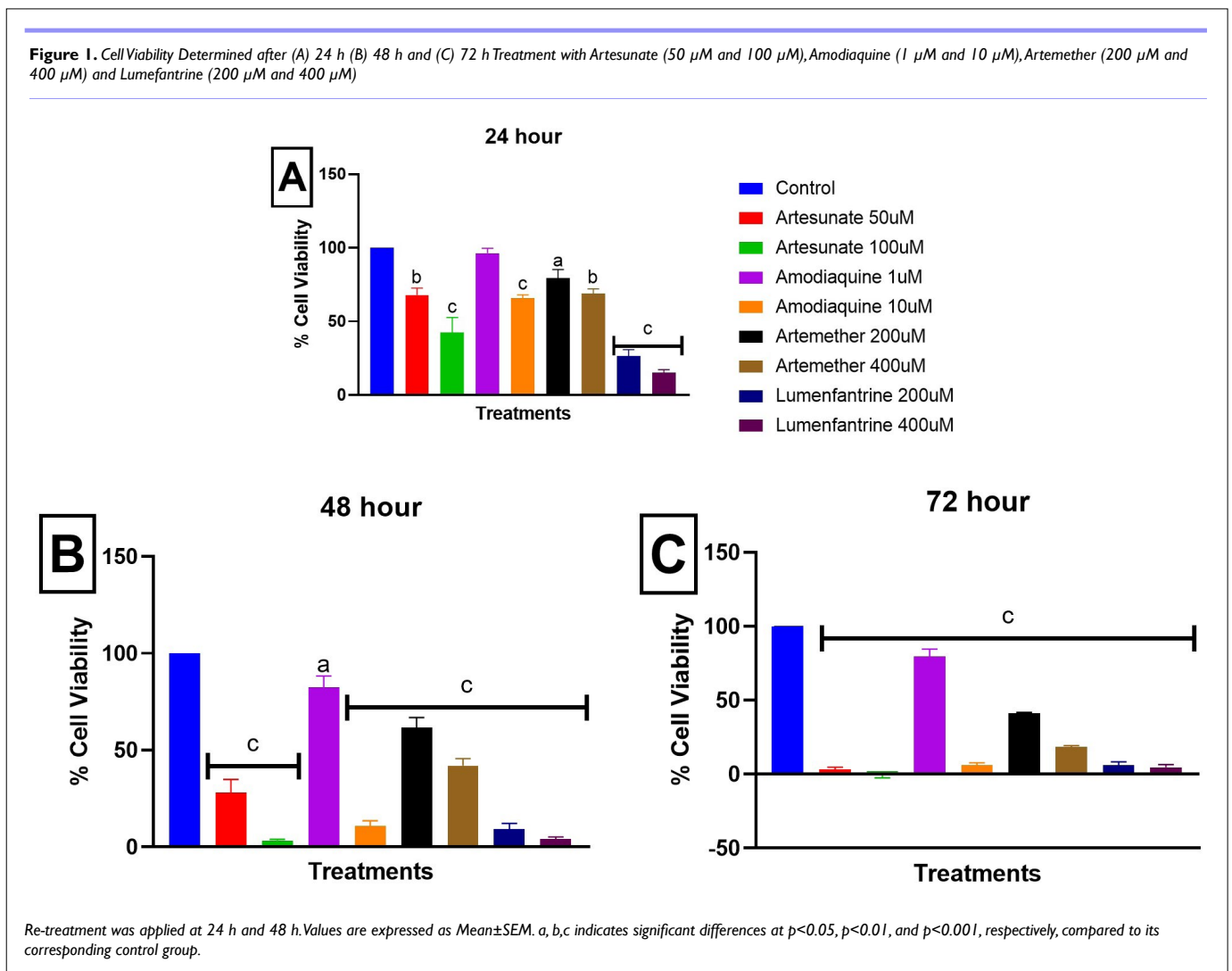
Exposure of cells to Artesunate 50  $\mu\text{M}$  lowered ROS by 40%, 60% ( $p < 0.001$ ), 87% ( $p < 0.001$ ) at 24 h, 48 h and 72 h, respectively compared to the control group; Treatments of cells with Artesunate 100  $\mu\text{M}$  reduced ROS further by 52% ( $p > 0.05$ ), 80% ( $p < 0.001$ ), 89% ( $p < 0.001$ ) at 24 h, 48 h and 72 h, respectively compared to control group (Figures 2 A, B and C).

Treatments of cells with Amodiaquine 1  $\mu\text{M}$  reduced ROS by 67%, 76% ( $p < 0.001$ ), 82% ( $p < 0.001$ ) for 24 h, 48 h, and

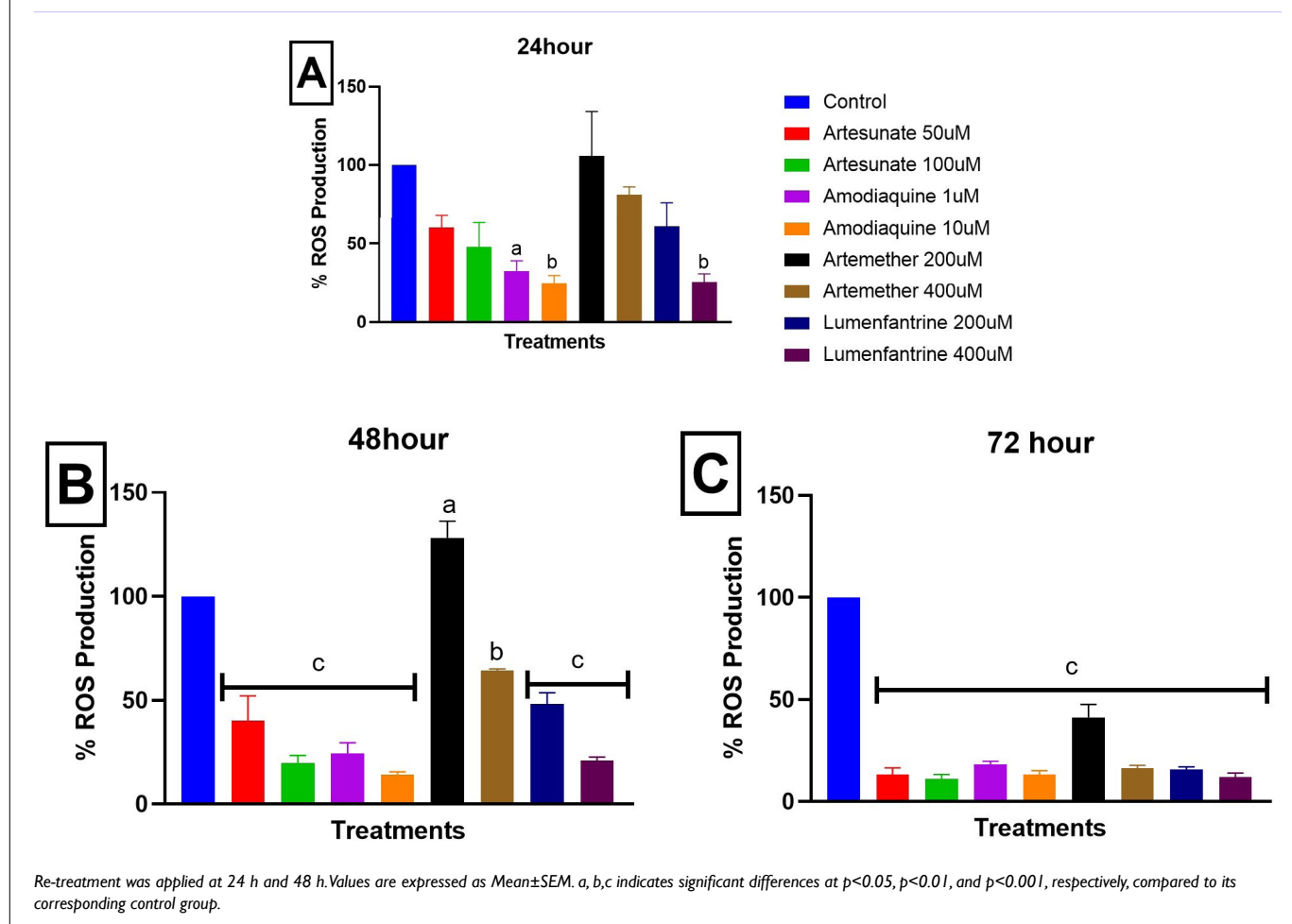
72 h respectively compared to the control group, while exposure of cells to Amodiaquine 10  $\mu\text{M}$  reduced ROS further by 75% ( $p < 0.01$ ), 86% ( $p < 0.001$ ), 87% ( $p < 0.001$ ) in 24, 48 and 72 h respectively compared to the control group (Figures 2 A, B and C).

Intriguingly, when compared to the control group, exposure of cells to artemether 200  $\mu\text{M}$  increased ROS by 6% and 28% ( $p < 0.05$ ) at 24 h and 48 h, respectively, but was reduced by 59% ( $p < 0.001$ ) after 72 h; While treatment of cells with artemether 400  $\mu\text{M}$  decreased ROS by 19%, 36% ( $p < 0.01$ ), and 84% ( $p < 0.001$ ) at 24 h, 48 h (Figures 2 A, B and C).

Treatment of cells with lumefantrine 200  $\mu\text{M}$  showed reduce ROS by 39%, 52% ( $p < 0.001$ ), and 84% ( $p < 0.001$ ) at 24 h, 48 h and 72 h respectively, compared to the control group, while Exposure of cells to lumefantrine 400  $\mu\text{M}$  recorded reduce ROS by 74% ( $p < 0.01$ ), 79% ( $p < 0.001$ ), 88% ( $p < 0.001$ ) at 24 h, 48 h and 72 h, respectively compared to the control group (Figures 2 A, B and C).



**Figure 2.** Reactive Oxygen Species (ROS) Production Determined after (A) 24 h (B) 48 h and (C) 72 h Treatment with Artesunate (50  $\mu$ M and 100  $\mu$ M), Amodiaquine (1  $\mu$ M and 10  $\mu$ M), Artemether (200  $\mu$ M, and 400  $\mu$ M) and Lumefantrine (200  $\mu$ M and 400  $\mu$ M)



## DISCUSSION

Research has shown that oxidative stress is that cause of artemisinin combination therapy (ACT) related liver damage in human and animal studies.<sup>15,16</sup> Therefore, this study investigated the cytotoxicity of repeated *in vitro* administration of artesunate, amodiaquine, artemether, and lumefantrine to liver cell lines. This mimics the clinical observation when individuals take anti-malarials prophylactically.

Treatment of HepG2 cells with these drugs resulted in significantly lower cell viability, with a decreasing trend from 24 h to 72 h (Figure 1). The mechanisms for cell death is still unclear and requires further investigation but hypotheses are linked to oxidative stress.<sup>12,17</sup> In addition, the anti-malarials mechanism of action is possibly *via* toxic hydroperoxide metabolites.<sup>7</sup>

Antimalarial drugs such as artesunate, amodiaquine, artemether, and lumefantrine are first metabolized in the liver *via* cytochrome P450 enzymes (CYP2A6, CYP3A4/5, CYP3A4, and CYP2B6). The phase 1 metabolite of artemether and artesunate is dihydroartemisinin. The metabolite of lumefantrine is desbutylbenflumetol, formed by the actions of CYP3A4/5, while the

metabolite of amodiaquine is monodesethylamine, formed by the actions of CYP3A4.<sup>1,18</sup> These metabolites inhibit the parasites growth and reproduction in red blood cells.<sup>1,18</sup> These metabolites contribute to the therapeutic effects of these drugs, but they can also cause adverse effects such as nausea, vomiting, and abdominal pain, headaches, jaundice and dizziness.<sup>1</sup> It is also possible that the parent drug and/or the metabolites possess toxic properties as evidenced in the following reports. The study by Idowu et al<sup>11</sup> found that treatment with artemether/lumefantrine in combination with albendazole and ivermectin, respectively, caused liver dysfunction and somatic mutations in uninfected rats through oxidative stress. This was demonstrated by significant increases in aspartate aminotransferase (AST) (35% and 67%), alanine aminotransferase (ALT) (39% and 59%), as well as a significant decreases in superoxide dismutase (SOD) (33% and 40%) and catalase (CAT) (32% and 22%). Silva-Pinto et al<sup>15</sup> found that patient treatment with artemisinin-lumefantrine for uncomplicated falciparum malaria resulted in an increase in ALT (75 *vs* 99 U/L,  $p<0.05$ ) and AST (64 *vs* 131 U/L,  $p<0.01$ ). However, the change in liver enzymes was not accompanied with any symptoms, suggesting that liver injury was not detected by drug treatment. Furthermore, the study undertaken by Adaramoye et al<sup>16</sup> found that treatment with either artemether-lumefantrine and artemether

alone, resulted in a decrease in the levels of glutathione (21% and 29%), glutathione S-transferase (51% and 64%), SOD (50% and 45%) and CAT (29%; and 20%), and an increase in lipid peroxidation (50% and 67%) in the liver of uninfected rats. These studies indicate increased oxidative stress occurs following drug treatment.

In the present study, increased ROS production was only observed in the lower dose (200  $\mu$ M) of artemether treatment after 48 h, but it is possible that a similar pattern occurred with artesunate, amodiaquine, artemether (at the higher dose of 400  $\mu$ M), and lumefantrine at earlier time points. The low ROS observed in artesunate, amodiaquine, artemether (at 400  $\mu$ M), and lumefantrine could be attributed to the initial high ROS production leading to cell death at 24 h, 48 h, and 72 h; hence decreased cell number could produce low ROS. Further studies, which are beyond the current short communication would measure ROS at earlier time points; live ROS cell imaging; markers of apoptosis and antioxidant enzyme activity. These parameters will provide further information as to the exact mechanism of cytotoxicity.

Studies have shown that artesunate exposure affects the proliferation of healthy liver cells by causing G0/G1 cell cycle arrest and apoptosis. This negative effect was attributed to intracellular ROS build-up.<sup>12</sup> Other studies also support ROS accumulation initiating apoptosis.<sup>17</sup> ROS production can cause local oxidative stress and accompanying adaptive responses, leading to adverse effects.<sup>19</sup> Some of the side effects of using artemether, lumefantrine include headaches, nausea, vomiting, anorexia, abdominal pain, and dizziness.<sup>20</sup> Amodiaquine is not used solely as a prophylaxis agent since it causes hepatitis and agranulocytosis, but is now combined with artesunate to reduce its side effects. However, patients still experience some adverse reactions, including overall body weakness, ear and eye problems, abdominal pain, and dizziness, cause by the amodiaquine constituent of the combined drug.<sup>21</sup>

## CONCLUSION

This brief communication has demonstrated that repeated administration of artesunate, amodiaquine, artemether, and lumefantrine resulted in a reduction in cell viability, possibly *via* oxidative stress. When utilising antimalarial drugs, it is important to consider any potential side effects of these medications on healthy, normal cells as this may cause tissue injury. As a result, repeated prophylactic administration of antimalarial drugs needs to be undertaken with extreme caution.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1. Audu D, Petagine L, Idowu OA, Patel VB, Idowu AB. Biomarkers of the toxic effects of chemotherapeutic agents: A focus on antimalarials. In: Patel VB, Preedy VR, Rajendram R, eds. *Biomarkers in Toxicology. Biomarkers in Disease: Methods, Discoveries and Applications*. NY, USA: Springer, Cham; 2023. doi: 10.1007/978-3-030-87225-0\_73-2
2. World Health Organization (WHO). World malaria report 2022. Web site. <https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022>. Accessed November 12, 2022.
3. Wangdi K, Furuya-Kanamori L, Clark J, et al. Comparative effectiveness of malaria prevention measures: A systematic review and network meta-analysis. *Parasit Vectors*. 2018; 11(1): 210. doi: 10.1186/s13071-018-2783-y
4. Aminake NM, Pradel G. Antimalarial drugs resistance in *Plasmodium falciparum* and the current strategies to overcome them. *Microbial Pathogens and Strategies for Combating them: Science, Technology and Education*. 2013; 1: 269-282.
5. Koko VS, Warsame M, Vonhm B, et al. Artesunate–amodiaquine and artemether–lumefantrine for the treatment of uncomplicated falciparum malaria in Liberia: In vivo efficacy and frequency of molecular markers. *Malar. J.* 2022; 21: 134. doi: 10.1186/s12936-022-04140-7
6. Kavishe RA, Koenderink JB, Alifrangis M. Oxidative stress in malaria and artemisinin combination therapy: Pros and cons. *FEBS Journal*. 2017; 284: 2579-2591. doi: 10.1111/febs.14097
7. Olliaro PL, Haynes RK, Meunier B, Yuthavong Y. Possible modes of action of the artemisinin-type compounds. *Trends Parasitol*. 2001; 17: 122-126. doi: 10.1016/S1471-4922(00)01838-9
8. Egwu CO, Augereau JM, Reybier K, Benoit-Vical F. Reactive oxygen species as the brainbox in malaria treatment. *Antioxidants*. 2021; 10: 1872. doi: 10.3390/antiox10121872
9. Owumi SE, Gbadegesin MA, Odunola OA, Adegoke AM, Uwaifo AO. Toxicity associated with repeated administration of artemether-lumefantrine in rats. *Environ Toxicol*. 2015; 30: 301-307. doi: 10.1002/tox.21907
10. Taylor WRJ, White NJ. Antimalarial drug toxicity: A review. *Drug Saf*. 2004; 27: 25-61. doi: 10.2165/00002018-200427010-00003
11. Idowu ET, Alimba CG, Olowu EA, Otubanjo AO. Artemether-Lumefantrine treatment combined with albendazole and ivermectin induced genotoxicity and hepatotoxicity through oxidative stress in Wistar rats. *Egypt J Basic Appl Sci*. 2015; 2: 110-119. doi: 10.1016/j.ejbas.2015.03.001
12. Yin S, Yang H, Zhao X, et al. Antimalarial agent artesunate induces G0/G1 cell cycle arrest and apoptosis via increasing intracellular ROS levels in normal liver cells. *Hum Exp Toxicol*. 2020; 39: 1681-1689. doi: 10.1177/0960327120937331
13. Ghazali R, Mehta KJ, Bligh SA, Tewfik I, Clemens D, Patel VB. High omega arachidonic acid/docosahexaenoic acid ratio



- induces mitochondrial dysfunction and altered lipid metabolism in human hepatoma cells. *World J Hepatol.* 2020; 12: 84-98. doi: [10.4254/wjh.v12.i3.84](https://doi.org/10.4254/wjh.v12.i3.84)
14. Eruslanov E, Kusmartsev S. Identification of ROS using oxidized DCFDA and flow-cytometry. *Methods Mol Biol.* 2010; 594: 57-72. doi: [10.1007/978-1-60761-411-1\\_4](https://doi.org/10.1007/978-1-60761-411-1_4)
15. Silva-Pinto A, Ruas R, Almeida F, et al. Artemether-lumefantrine and liver enzyme abnormalities in non-severe *Plasmodium falciparum* malaria in returned travellers: A retrospective comparative study with quinine-doxycycline in a Portuguese centre. *Malar J.* 2017; 16: 1-5. doi: [10.1186/s12936-017-1698-y](https://doi.org/10.1186/s12936-017-1698-y)
16. Adaramoye OA, Osaimoje DO, Akinsanya AM, Nneji CM, Fafunso MA, Ademowo OG. Changes in antioxidant status and biochemical indices after acute administration of artemether, artemether-lumefantrine and halofantrine in rats. *Basic Clin. Pharmacol. Toxicol.* 2008; 102: 412-418. doi: [10.1111/j.1742-7843.2008.00211.x](https://doi.org/10.1111/j.1742-7843.2008.00211.x)
17. Efferth T, Glaisi M, Merling A, Krammer PH, Li-Weber M. Artesunate induces ROS-mediated apoptosis in Doxorubicin-resistant T leukemia cells. *PLoS One.* 2007; 2: e693. doi: [10.1371/journal.pone.0000693](https://doi.org/10.1371/journal.pone.0000693)
18. Kerb R, Fux R, Mörike K, et al. Pharmacogenetics of antimalarial drugs: effect on metabolism and transport. *Lancet Infect Dis.* 2009; 9: 760-774. doi: [10.1016/S1473-3099\(09\)70320-2](https://doi.org/10.1016/S1473-3099(09)70320-2)
19. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. *J Toxicol.* 2012; 2012: 645460. doi: [10.1155/2012/645460](https://doi.org/10.1155/2012/645460)
20. Alkadi HO. Antimalarial drug toxicity: A review. *Chemotherapy.* 2007; 53: 385-391. doi: [10.1159/000109767](https://doi.org/10.1159/000109767)
21. Adisa R, Fakeye T, Dike D. Evaluation of adverse drug reactions to artemisinin-based combination therapy in a Nigeria University Community. *Tropical Journal of Pharmaceutical Research.* 2008; 7: 937. doi: [10.4314/tjpr.v7i2.14680](https://doi.org/10.4314/tjpr.v7i2.14680)