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Original Research Article

Dose and time-dependent effect of orange juice on the antimalarial efficacy of artemether-lumefantrine, haematological, and antioxidant parameters

John K. Iregbu[1], Jessica C. Iregbu[2], Gabriel C. Edeh[3]*, Daniel L. Ajaghaku[4], Christian C. Nwoye[1], Ezinneamaka M. Ashibogwu[1], Chukwuemeka C. Njoku[5], Chidera M. Ugwuegbu[6], Bostock C. Okafor[7], Obinna H. Tony-Okpalaobi[8], Chibuikem E. Okparaeke[1]

[1]Department of Medicine and Surgery, Faculty of Medicine, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[2]Department of Pharmacy, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria
[3]Department of Haematology, Babcock University Teaching Hospital, Ilishan-Remo, Ogun State, Nigeria
[4]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria
[5]Department of Obstetrics and Gynaecology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria
[6]Department of Surgery, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria
[7]Department of Medicine and Surgery, College of Health Sciences, University of Abuja, Federal Capital Territory, Nigeria
[8]Department of Internal Medicine, Federal Medical Centre, Jabi, Abuja, Nigeria

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***Correspondence:**
Dr. Gabriel C. Edeh,
E-mail: edehgabrielchidera@gmail.com

ABSTRACT

Background: Orange juice (OJ) contains ascorbic acid, which has antioxidant properties that would be expected to protect against haemolytic effects and possibly interfere with the antimalarial mechanism of the artemisinin-based combination therapy. This study was designed to investigate the dose and time-dependent effect of OJ on the anti-malarial efficacy of artemether-lumefantrine (AL).
Methods: Malaria was induced by inoculating plasmodium berghei parasite through the intraperitoneal route to albino mice. The infection was allowed to establish for 3 days, followed by treatment with AL alone, and its combination with graded doses of OJ. Separate doses of OJ were also administered to different groups.
Results: Treatment with AL alone produced total parasite clearance (100%) in days 3 and 4 post-treatment. Co-administration of AL with graded doses of OJ showed significant ($p<0.05$) increase in parasitemia at OJ combination doses of 10, 8, and 6 ml/kg. Unlike antagonistic combination interaction exhibited by higher combination doses of OJ, lower combination doses of 4 and 2 ml/kg potentiated the antiplasmodial activity of AL. Compared to vehicle (malaria) control group, a significant ($p<0.05$) increase in PCV was achieved at combination OJ doses of 10, 8, 6, and 4 ml/kg. Combination doses of OJ at 10, 8, 6 ml/kg restored the catalase enzyme activity to the level that exist in uninfected naïve control group.
Conclusions: OJ showed both antagonistic and beneficial interactions with AL at higher and lower doses, respectively. Combination of OJ with AL also has a beneficial effect in reducing both parasite and AL-induced oxidative stress.

Keywords: Ascorbic acid, Arthemether/lumefantrine, Malaria, Orange juice, Anti-malarial, Dose and time dependent effects

INTRODUCTION

Malaria is a potentially fatal disease triggered by protozoan parasites of the *Plasmodium* genus. Among the five species identified to infect humans, including *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi,* the latter, though primarily zoonotic, has emerged as a notable human pathogen in several parts of Southeast Asia. *Plasmodium falciparum* remains the deadliest malarial parasite and is predominantly found in the African continent.[1] The disease is transmitted when an infected female *Anopheles* mosquito bites a human, allowing the parasites to enter the bloodstream and subsequently localize in the liver, where they undergo maturation and multiplication. Malaria continues to rank among the leading causes of disease and mortality globally, especially in low-income nations, affecting primarily children under 5 years of age, travellers, immunocompromised individuals, and pregnant women. According to the Centers for Disease Control and Prevention (CDC), the global malaria burden in 2022 was estimated at 249 million cases and about 608,000 fatalities.[2] The World Health Organization (WHO) indicates that sub-Saharan Africa bears the brunt of this burden, contributing to 94% of global cases and 95% of malaria-related deaths, with Nigeria alone accounting for approximately 26.8% of total cases.[3] National statistics estimate that Nigeria experiences around 68 million malaria cases and 194,000 associated deaths each year.[3]

Antimalarial treatment has a long history, with quinine being one of the earliest effective remedies dating back to the 17th century. In the 1900s, the development of synthetic drugs such as chloroquine, mefloquine, and primaquine marked significant progress. However, the emergence of drug-resistant *Plasmodium* strains soon began to undermine the efficacy of these therapies. This necessitated the discovery of a new category of antimalarial drugs, which was artemisinins and their derivatives. Despite their high efficacy, artemisinins have short half-lives, which prompted the formulation of combination therapies. To sustain therapeutic levels and curb resistance, artemisinin-based combination therapies (ACTs) were introduced, combining fast-acting artemisinin compounds with longer-lasting partner drugs.

One of the most widely used ACTs is artemether-lumefantrine, which unites the fast-acting artemether with lumefantrine to achieve sustained parasite clearance. This combination has significantly improved malaria treatment outcomes and has been widely adopted as a first-line therapy in many endemic regions. Nonetheless, optimizing its effectiveness remains critical, especially in areas experiencing rising drug resistance. An often overlooked variable in drug efficacy is dietary influence, which can alter a drug's pharmacokinetic and pharmacodynamic profile and, in turn, impact therapeutic performance.

Artemether exerts its antimalarial effect by generating reactive oxygen species (ROS) through interaction with blood constituents, which damages the parasite and alleviates clinical symptoms. Lumefantrine complements this action by clearing residual parasites, reducing the overall parasitic burden. In some treatment settings, these drugs are co-administered with vitamin C (ascorbic acid), which is primarily known for its antioxidant functions and its ability to alleviate oxidative stress and anemia, both of which are associated with malaria. Laboratory findings suggest that vitamin C can enhance the effects of certain antimalarial compounds, such as exifone.[4] Furthermore, individuals with malaria often exhibit reduced plasma concentrations of vitamin C, thus supporting its supplemental use during treatment.[5] Nonetheless, some experts caution against using ascorbic acid-rich products such as fruit juices during antimalarial therapy, due to potential interference with drug activity.[6] This caution is further supported by studies demonstrating that antioxidants may impair the efficacy of drugs that rely on oxidative mechanisms to kill the parasite.[7,8]

Although artemether-lumefantrine remains an essential part of malaria treatment, cases of therapeutic failure continue to be reported. These failures may arise from the emergence of drug-resistant parasite strains or other external factors affecting drug performance. While antioxidants can be helpful in managing oxidative damage from both malaria and drug exposure, they may also reduce the efficacy of medications like artemether, which depend on oxidative stress to eliminate parasites. Because artemether operates through ROS-mediated mechanisms, concurrent intake of antioxidant-rich substances, either through supplements or dietary sources, could impair its function. Orange fruit, widely consumed for its nutritional value, contains significant quantities of ascorbic acid and is often taken during illness for its perceived health benefits. In malaria-endemic regions, it is plausible that individuals undergoing treatment with artemether-lumefantrine might consume orange juice alongside their medication, often without recognizing the potential for pharmacological interactions.

The gap in existing knowledge on the nature of drug-food interaction between varying doses of orange juice and the therapeutic dose of artemether lumefantrine represents a critical obstacle in the efforts towards optimizing malaria treatment regimens and combating the development of resistance, potentially compromising patient outcomes, safety, and global control efforts. This study therefore explored the comparative effect of plasmodium berghei clearance rate of artemether lumefantrine, as well as on the haematological and anti-oxidative markers in artemether lumefantrine-treated animals alone, and in combination with different doses of orange juice.

Investigating dose and time-dependent interaction of orange juice on artemether-lumefantrine effectiveness is crucial for informing treatment protocols and advancing malaria management strategies, particularly in resource-limited settings where the disease burden is high.

METHODS

Study location

This study was carried out at the School of Pharmacy, Nnamdi Azikiwe University, Anambra State, Nigeria.

Study period

This study took place from February 2024 to March 2024.

Type of study

This was a controlled in vivo laboratory experiment evaluating the dose- and time-dependent interaction between orange juice and artemether-lumefantrine in a murine malaria model.

Plant collection and extraction

Fresh orange fruits were bought from Ogbete market, Enugu. The fruits (2 kg) were washed with clean tap water and rinsed with distilled water. The peel was removed and the fruit extracted with an electronic juice extractor (Binatone JE-580). The juice was further filtered using a vacuum funnel. The fruit juice extract was stored in the refrigerator at 2ºC.

Animals

Swiss Albino mice weighing 24–30 g and aged 7-8 weeks of both sexes were used for this study. All the animals were obtained from the Animal House of the Department of Pharmacology, Enugu State University of Science and Technology, Enugu State, Nigeria. The animals were allowed to acclimatize for one week prior to the commencement of the study. Food and water were provided *ad libitum*. All animal experiments were conducted in compliance with the National Institute of Health (NIH) guide for care and use of laboratory animals, and approved by the institution's animal ethical committee with approval number ESUT/FPS/PHA/2024/431.[9]

Parasite

The *Plasmodium berghei* used for the study was obtained from the Nigeria Institute of Medical Research, Lagos, Nigeria. The parasite was of the ANKA strain with a chloroquine resistance phenotype. It was maintained by serial passage in mice in our laboratory.

Iodometric determination of ascorbic acid by titration

1% starch indicator solution

Soluble starch (0.5 g) was added to 50 ml of near-boiling distilled water. The solution was well-mixed and allowed to cool before use.

Iodine solution

Potassium iodide (KI, 5 g) and potassium iodate (KIO_3, 0.268 g) were dissolved in 200 ml of distilled water. Sulphuric acid (30 ml, 3M) was added to the preparation and poured into a 500 ml graduated cylinder. This was further diluted to a final volume of 500 ml with distilled water. The resulting solution was well mixed and transferred to a labelled 600 ml beaker.

Vitamin C standard solution

Ascorbic acid (0.250 g) was dissolved in 100 ml of distilled water. The resulting solution was diluted to 250 ml with distilled water in a labelled volumetric flask.

Standardizing solutions

A 25 ml volume of vitamin C solution was added to a 125 ml Erlenmeyer flask. Starch solution (10 drops, 1%) was added as the indicator. The burette was rinsed with a small volume of the iodine solution and then filled to the 25 ml mark. The vitamin C solution was titrated with the iodine solution until the first sign of blue colour that persisted after 20 seconds of swirling the solution. This was taken as the endpoint. The volume of the iodine solution required to achieve the endpoint was calculated as the difference between the starting volume and the final volume. The titration was done in triplicate, and the mean value calculated.

Titrating the orange juice

A 25 ml of orange juice was added to a 125 ml Erlenmeyer flask. This was titrated until the endpoint was reached. The titration was also done in triplicate and mean calculated.

Calculating ascorbic acid content

The ascorbic acid content in g/ml was calculated using the following formula.

$$Ascorbic\ acid\ content\ in \frac{g}{ml} = \frac{\frac{A}{0.250\ g\ (Vit\ C\ standard\ solution)}}{\frac{B}{ascorbic\ acid\ content\ of\ the\ orange\ juice}}$$

Where A is the titrant required for the standard solution and B the titrant required for the orange juice.

Experimental design (animal grouping and dosing)

The albino mice used for the experiment were randomly divided into thirteen groups that consisted of five animals per group - group 1: given 10 ml/kg distilled water as naive control group (mice not inoculated with *P. berghei*), group 2: 10 ml/kg 5% Tween 80 as vehicle control group (mice

inoculated with *P. berghei*), group 3: orally administered 2.3/13.7 mg/kg b. w. Artemether lumefantrine, group 4: orally administered 2.3/13.7 mg/kg b. w. artemether lumefantrine + 10 ml/kg b. w. orange juice, group 5: orally administered 2.3/13.7 mg/kg b. w. artemether lumefantrine + 8 ml/kg b. w. orange juice, group 6: orally administered 2.3/13.7 mg/kg b. w. artemether lumefantrine + 6 ml/kg b. w. orange juice, group 7: orally administered 2.3/13.7 mg/kg b. w. artemether lumefantrine + 4 ml/kg b. w. orange juice, group 8: orally administered 2.3/13.7 mg/kg b. w. artemether lumefantrine + 2 ml/kg b. w. orange juice, group 9: orally administered 10 ml/kg b. w. orange juice, group 10: orally administered 8 ml/kg b. w. orange juice, group 11: orally administered 6 ml/kg b. w. orange juice, group 12: orally administered 4 ml/kg b. w. orange juice, and group 13: orally administered 2 ml/kg b. w. orange juice.

Inoculation of mice

Albino mice previously infected with *P. berghei* and having a parasitemia level of 50–60% were used as donors. Blood samples were collected from the donor mice via retro-orbital plexus into heparinized tubes. The blood was then diluted with normal saline (0.9%) based on parasitemia of the donor mice and the red blood cells (RBC) count in such a way that 1 ml blood contained 5×10^7 infected erythrocytes. Thereafter, 0.2 ml blood, containing 1×10^7 *P. berghei* infected erythrocytes, was injected through intraperitoneal (ip) route for each mouse except the naïve control group.

Treatment of infected animals

After 72 hours of infection with the parasite, parasitemia was confirmed in the animals prior to treatment with their respective doses as described in the animal grouping and dosing section above. Treatment was done for 4 days.

On the 5th day, blood samples were collected from the animals through retro-orbital plexus into plain tubes and also in anticoagulant tubes. The blood in the plain tubes were centrifuged (3000 g for 15 min) for the separation of serum. The serum was used for biochemical assays. Thin blood film was immediately prepared directly from the whole blood while bleeding.

Determination of parasitemia

Parasitemia determination was done by counting the number of infected RBCs (a minimum of three fields per slide) using a light microscope (MB23 0 T, China) with an objective lens magnification power of 100x. Percent parasitemia and percent inhibition were calculated using the modified Peters and Robinson formula.

$$\%\ Parasitemia = \frac{Number\ of\ parasitized\ RBC}{Total\ number\ of\ RBC\ ocunt} \times 100$$

$$\%\ Plasmodium\ clearance = \frac{Mean\ parasite\ count\ before\ treatment - Mean\ parasite\ count\ after\ treatment}{Mean\ parasite\ count\ before\ treatment} \times 100$$

Determination of packed cell volume

A microhematocrit centrifuge (Hettichhaematokrit, Germany) was used. Centrifugation was done at 12,000 rpm for 5 minutes, after blood was collected from the tail of each mouse using heparinized capillary tubes.

PCV was determined using the following calculation.

$$PCV = \frac{Volume\ of\ erythrocytes\ in\ a\ given\ volume\ of\ blood}{Total\ blood\ volume} \times 100$$

Haemoglobin quantification

Haemoglobin content of the whole blood was quantified using Drabkin's reagent ($K_3Fe(CN)_6$ 200 mg/l, KCN 50 mg/l, $NaHCO_3$ 1 g/l, pH 8.6). The procedure is based on the oxidation of haemoglobin to methemoglobin in the presence of alkaline potassium ferricyanide. The methemoglobin reacts with potassium cyanide to form cyanmethemoglobin. The colour intensity measured at 540 nm is proportional to the total haemoglobin concentration.

To all tubes – blank and sample, 5 ml of the Drabkin's solution was added followed by 20 ul of whole blood added to the sample tubes while distilled water was added to the blank tube. The tubes were mixed, allowed to stand for 15 minutes at room temperature. Absorbance of tests was read at 540 nm after blanking, and total haemoglobin concentration (mg/ml) was derived from the calibration curve of cyanmethemoglobin.

Determination of antioxidant enzyme activity

Serum catalase activity was estimated by visible light method as described by Weydert and Cullen using catalase assay kit (Elabscience Biotechnology Co. Ltd., China) while serum superoxide dismutase activity (SOD) was estimated by hydroxylamine method as described by Weydert and Cullen using SOD assay kit (Elabscience Biotechnology Co. Ltd., China).[10]

Determination of reduced glutathione concentration

In order to estimate the level of reduced glutathione in serum, the method published by Rahman et al was followed.[11] In most cases, the reduced form of glutathione contains the majority of cellular non-protein sulfhydryl groups. This approach is based on the production of a fairly stable yellow color when sulfhydryl compounds are treated with 5,5-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent). Ellman's reagent reacts with reduced glutathione

to produce 2-nitro-5-thiobenzoic acid, a chromophoric compound having a molar absorption of 412 nm.

Determination of the lipid peroxidation (LPO) in serum

The level of thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) production was measured in serum by the modified method as described by Draper and Hadley.[12] The serum (50 µl) was deproteinized by adding 1 ml of 14% trichloroacetic acid and 1 ml of 0.6% thiobarbituric acid. The mixture was heated in a water bath for 30 min to complete the reaction and then cooled on ice for 5 min. After centrifugation at 2000 g for 10 min, the absorbance of the coloured product (TBARS) was measured at 535 nm with a UV spectrophotometer. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56×10^5 mol/l/cm) using the formula, A=ΣCL, where A=absorbance, Σ=molar coefficient, C=concentration, and L=path length. The results were expressed in nmol/mg of protein.

Determination of serum total antioxidant capacity

Total antioxidant capacity of the serum was determined using the ABTS radical cation decolorization assay method as described by Rice-Evans.[13] The assay using Sigma-Aldrich (USA) antioxidant assay kit (Catalog number CS0790) was based on the ability of serum antioxidants to inhibit the formation of ferryl myoglobin radical from metmyoglobin and hydrogen peroxide which oxidizes the 2, 2-Azino-di-3-ethylbenzthiazoline sulphonate (ABTS) to produce a radical cation that can be measured spectrophotometrically at 405 nm. The assay was calibrated with Trolox and total antioxidant capacity expressed in terms of Trolox equivalents (mM).

Statistical analysis

Results were presented as mean±standard error of mean (SEM). The analysis of variance in the outcome of the treatment (one-way ANOVA) was done using statistical package for social science (SPSS, version 20). Post hoc multiple comparison analysis was done using Turkey's test.

RESULTS

Ascorbic acid content of orange juice

Iodometric titration of ascorbic acid content of orange juice revealed 0.64±0.01 mg/ml concentration.

Effect of orange juice alone and in combination with artemether lumefantrine on parasitemia

No significant ($p>0.05$) difference in parasitemia was recorded across groups at the pre-treatment stage of the study. However, comparism with vehicle control group revealed that treatment groups both in combination (orange juice and artemether lumefantrine) and separately (artemether lumefantrine) showed significant ($p<0.05$) reduction in percentage parasitemia from day 1 post-treatment to day 4 post-treatment. Treatment with artemether lumefantrine (AL) alone reduced parasitemia to 8% in day 1 post treatment and less than 0.8 in day 2 (Figure 1). No parasitemia was recorded in the AL alone treated group from day 3 post-treatment. Total parasite clearance (100%) was recorded in day 3 and 4 post-treatment (Figure 2).

Co-administration of AL with graded doses of orange juice (OJ) showed significant ($p<0.05$) increase in parasitemia at OJ combination doses of 10, 8 and 6 ml/kg. Combinations of these doses of OJ with artemether lumefantrine led to decrease in the rate of AL alone mediated parasite clearance. At day 4 post-treatment, combination dose of 10 ml/kg of OJ and AL was unable to produce total parasite clearance while 6 ml/kg and 8 ml/kg combination doses showed total clearance from day 3 and on day 4 post-treatment time intervals respectively.

Unlike antagonistic combination interaction exhibited by higher combination doses of OJ, lower combination doses of 4 and 2 ml/kg potentiated the antiplasmodial activity of AL. combination dose of OJ at 2 ml/kg produced similar reduction in parasitemia just like AL alone on day 1 and 2 post-treatment time intervals with total parasite clearance occurring from day 3 post-treatment just like AL alone. At combination dose of 4 ml/kg of OJ, significant ($p<0.05$) improvement in parasite clearance was recorded more than that produced by AL alone on day 1. Improved plasmodium clearance rate was also observed with total plasmodium clearance occurring on day 2 unlike AL alone that produced total clearance from day 3.

Treatment with graded doses of OJ alone did not bring about reduction in parasitemia however, the rate of parasite turnover was dose dependently reduced from day 1 to day 4 compared to vehicle (malaria) control group (Figures 2 and 3). Significant ($p<0.05$) reduction was recorded at 10, 8 and 6 ml/kg from day 1 while 4 and 2 ml/kg produced significant effect from day 2 and 3 respectively.

Effect of orange juice alone and in combination with artemether lumefantrine on haematological parameters

Both malaria and AL treatment brought about significant ($p<0.05$) reduction in packed cell volume (PCV) and haemoglobin (Hb) concentration compared to naïve (uninfected) control group (Figure 4). Combination of OJ at all tested doses except 10 ml/kg and at all tested separate doses failed to restore PCV to uninfected status as demonstrated by significant ($p<0.05$) reduction in PCV in all these groups compared to naïve control group.

Compared to vehicle (malaria) control group, significant ($p<0.05$) increase in PCV was achieved at combination OJ doses of 10, 8, 6 and 4 ml/kg. similarly, these doses of OJ

when given alone produced significant ($p<0.05$) increase in PCV compared to vehicle control group. Compared to AL alone treated group, combination with OJ at 10, 8 and 6 ml/kg significantly ($p<0.05$) improved PCV just like these doses of OJ when given alone.

Similar degree of reduction in Hb was recorded in vehicle (malaria) control group and AL treated group with no significant ($p>0.05$) difference occurring between these groups. Combination of OJ at 10, 8, 6 and 4 ml/kg with AL brought about significant ($p<0.05$) improvement in Hb concentration just like these concentrations of OJ given alone compared to both vehicle and AL alone control groups.

Effect of orange juice alone and in combination with artemether lumefantrine on antioxidant parameters

Malaria infection brought about significant ($p<0.05$) reduction in serum catalase enzyme activity just like treatment with AL alone when compared to the uninfected naïve control group. Combination doses of OJ at 10, 8, 6 ml/kg brought about restoration of catalase enzyme activity to the level that exist in uninfected naïve control group with no-significant ($p>0.05$) difference occurring between these groups compared to the vehicle (malaria) control group (Figure 5). Significant ($p<0.05$) improvement in catalase enzyme activity were recorded at OJ combination doses of 10, 8, 6 and 4 ml/kg.

It was also observed that superoxide dismutase (SOD) enzyme activity and serum reduced glutathione (GSH) were also significantly ($p<0.05$) reduced in the vehicle (malaria) and AL alone control groups compared to naïve (uninfected) control group (Figures 6 and 7). OJ both in combination and alone did not restore the SOD and GSH to the uninfected state. However, combination doses of OJ at 10, 8, 6, and 4 ml/kg as well as when given alone produced significant ($p<0.05$) improvement in SOD enzyme activity when compared to vehicle and AL alone control groups. Similarly, significant ($p<0.05$) improvement in GSH was also achieved at combination doses of 10 and 8 ml/kg of orange juice as well as when these doses of OJ were given alone compared to vehicle and AL alone control groups.

Just like the vehicle (malaria) control group, treatment with AL alone brought about increase in lipid peroxidation evidenced by significant ($p<0.05$) increase in malondialdehyde – a by-product of lipid peroxidation compared to naïve (uninfected) control group (figure 8). The increase in lipid peroxidation was significantly ($p<0.05$) inhibited by treatment with combination doses of OJ at 10, 8 and 6 ml/kg. No significant ($p>0.05$) difference existed between these combination doses and naïve (uninfected) control group. Compared to vehicle and AL alone control groups, OJ at all doses both in combination with AL and alone produced significant ($p<0.05$) reduction in serum malondialdehyde concentration.

Similarly, significant ($p<0.05$) reduction in serum total antioxidant was observed in vehicle (malaria) control group and AL alone control group compared to naïve (uninfected) control group (Figure 9). Treatment with OJ both in combination and separately at all tested doses were unable to restore serum total antioxidant to value present in naïve control group. However, when compared to vehicle and AL alone control groups, OJ at all tested doses both in combination with AL and separately showed significant ($p<0.05$) improvement.

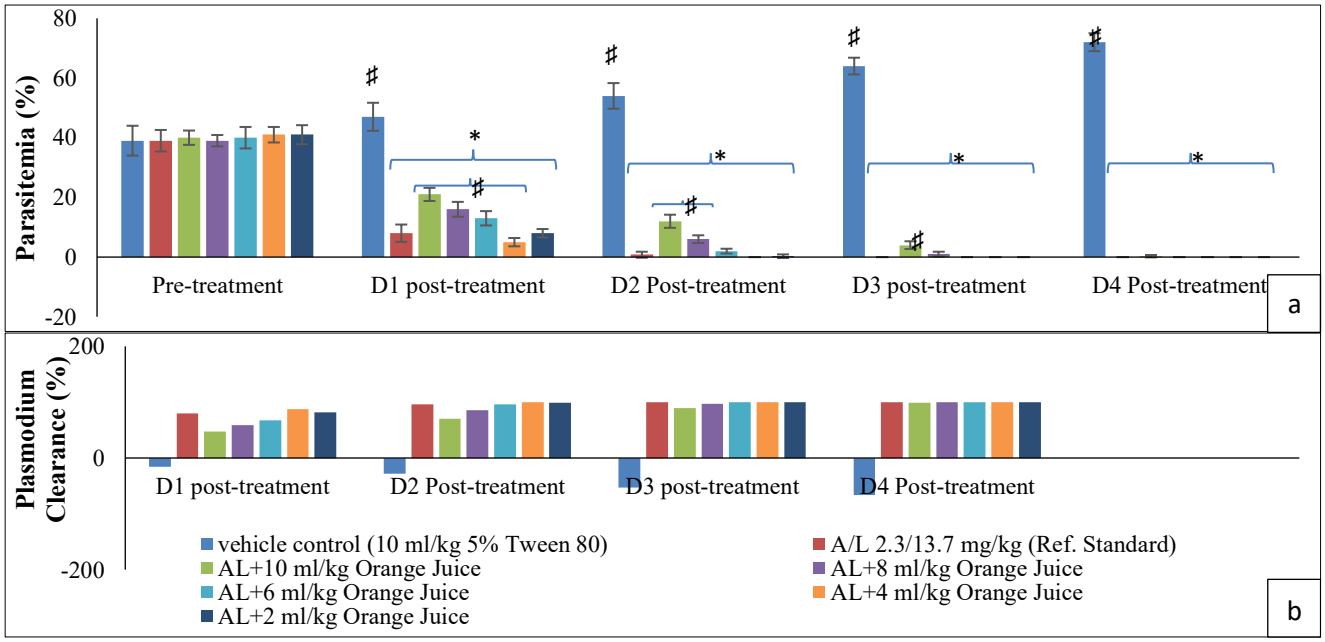

Figure 1 (a and b): Combination effect of artemether lumefantrine and orange juice on serum parasitemia and parasite clearance.

*P<0.05 compared to vehicle control; ♯p<0.05 compared to artemether lumefantrine alone.

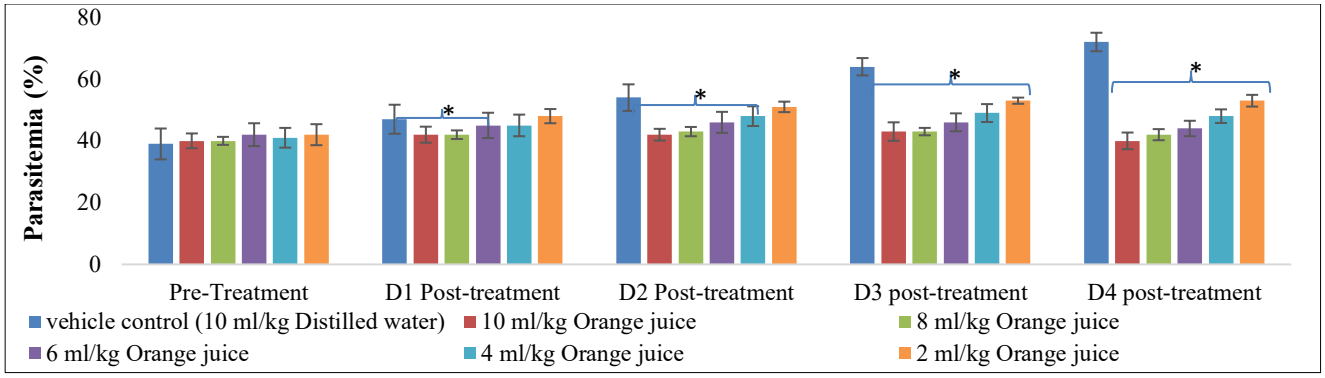

Figure 2: Dose-time response effect of orange juice on parasitemia.

*P<0.05 compared to vehicle control.

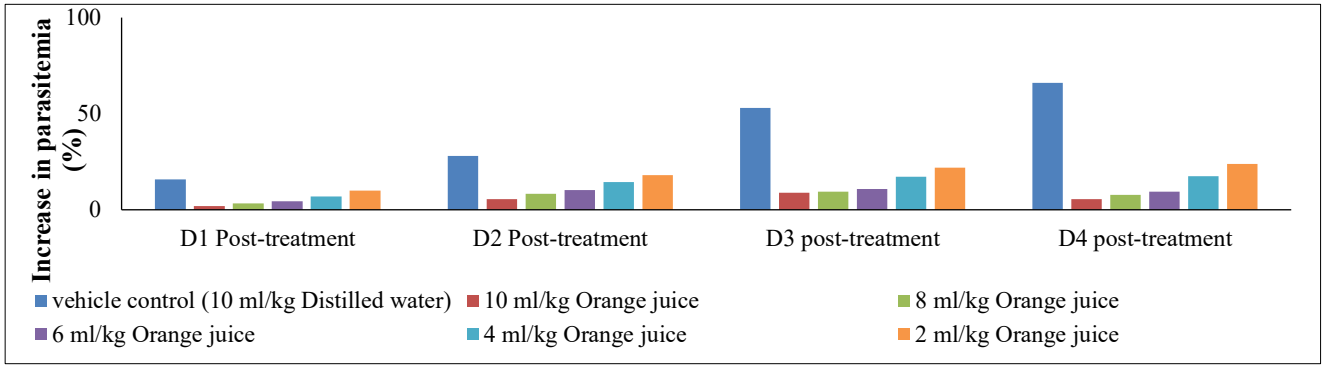

Figure 3: Effect of orange juice on percentage increase in parasitemia.

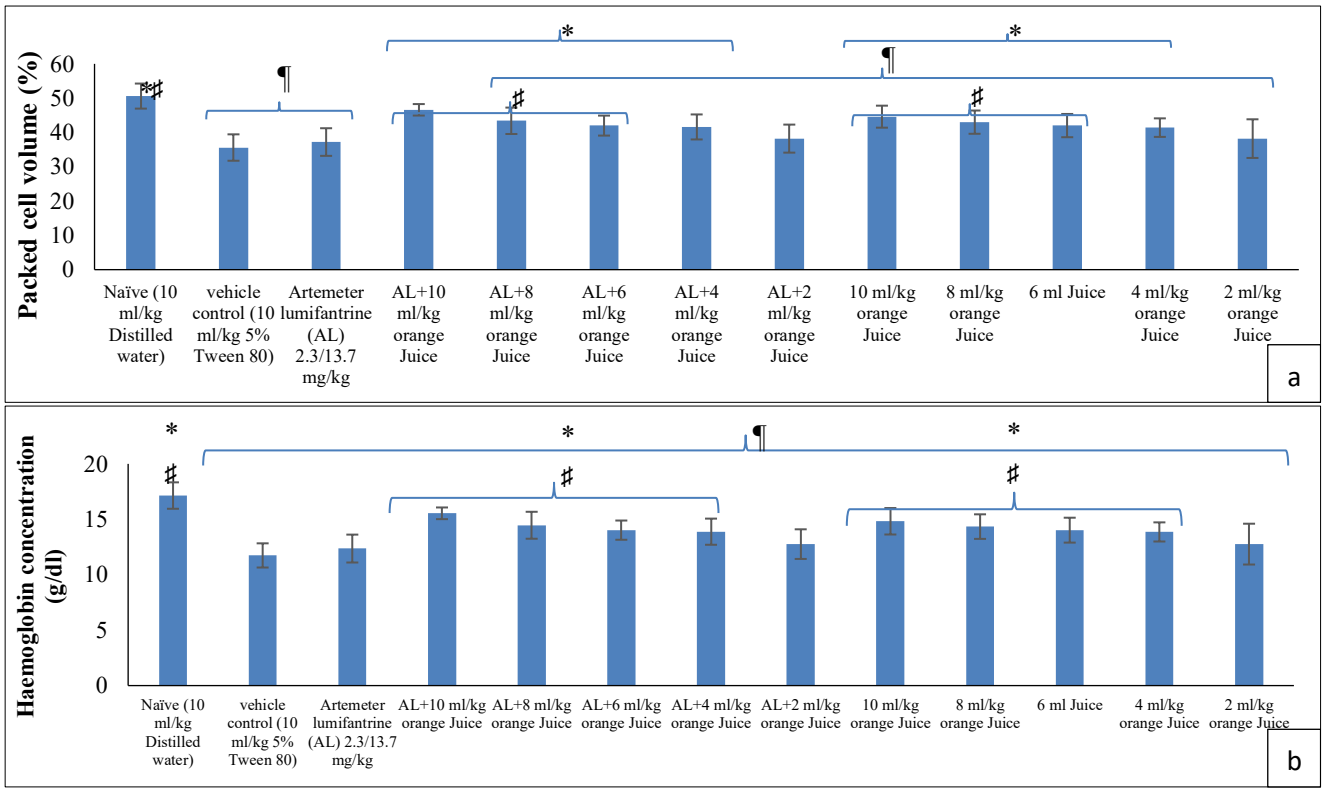

Figure 4 (a and b): Effect of artemether lumefantrine combination with orange juice on packed cell volume and haemoglobin concentration.

* P<0.05 compared to vehicle control; ♯ p<0.05 compared to artemether lumefantrine alone; ¶ p<0.05 compared to naïve control.

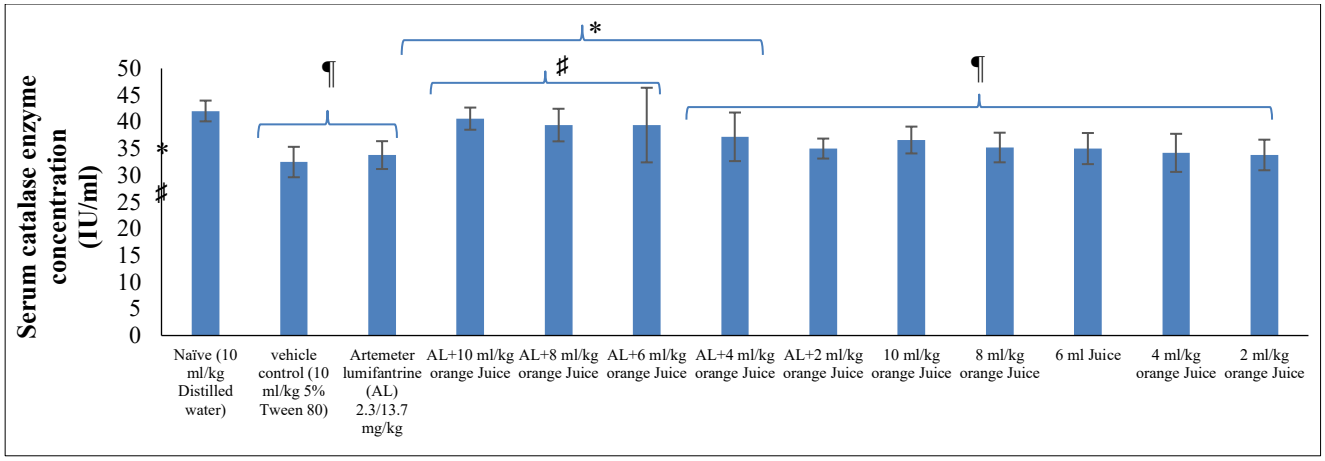

Figure 5: Effect of artemether lumefantrine combination with orange juice on serum catalase enzyme concentration.

* P<0.05 compared to vehicle control; ♯ p<0.05 compared to artemether lumefantrine alone; ¶ p<0.05 compared to naïve control.

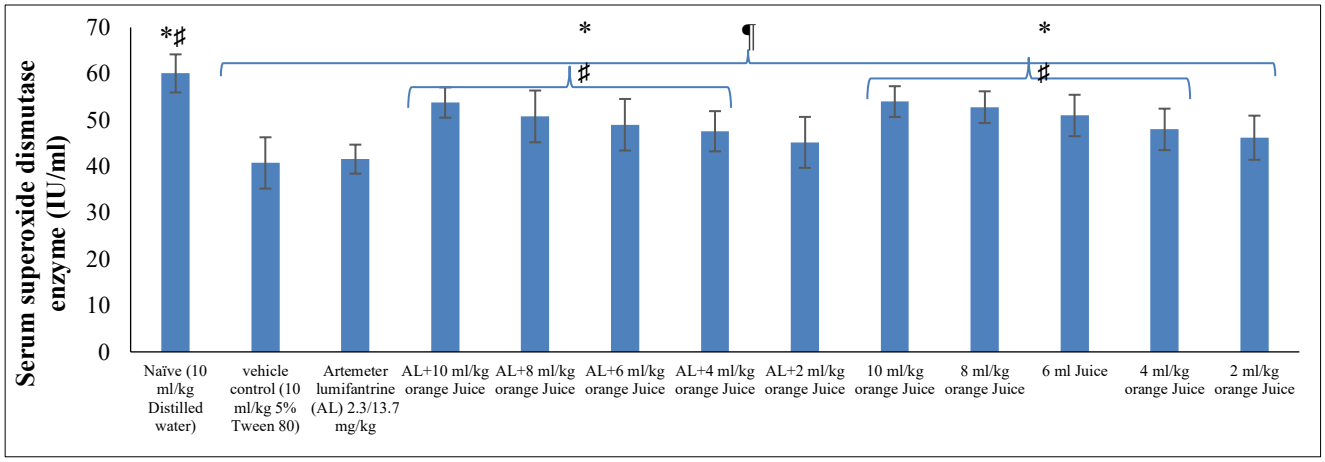

Figure 6: Effect of artemether lumefantrine combination with orange juice on serum superoxide dismutase enzyme concentration.

* P<0.05 compared to vehicle control; ♯ p<0.05 compared to artemether lumefantrine alone; ¶ p<0.05 compared to naïve control.

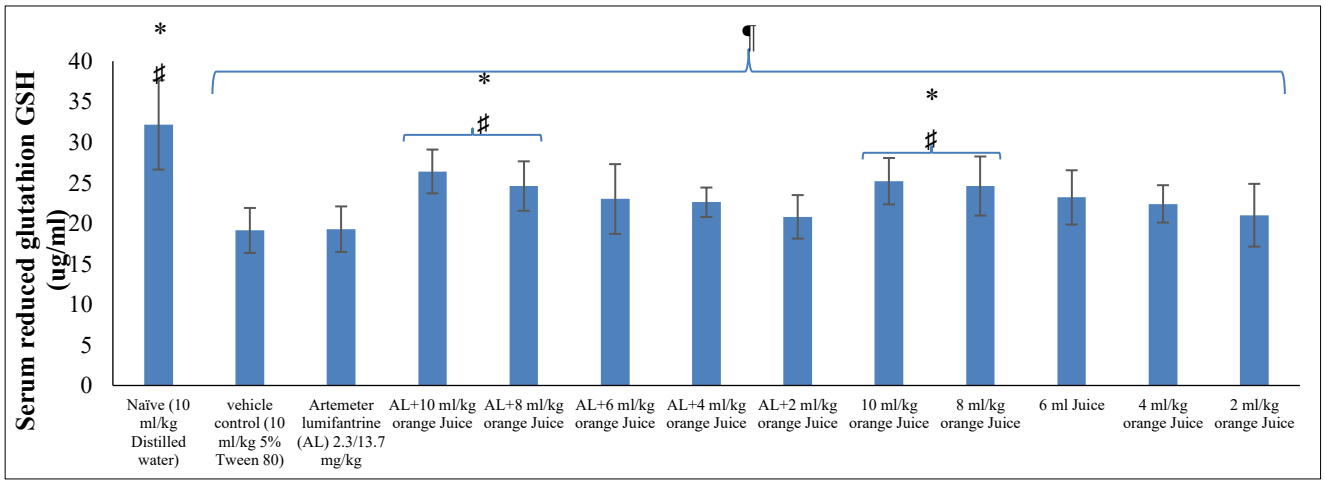

Figure 7: Effect of artemether lumefantrine combination with orange juice on serum reduced glutathione concentration.

* P<0.05 compared to vehicle control; ♯ p<0.05 compared to artemether lumefantrine alone; ¶ p<0.05 compared to naïve control.

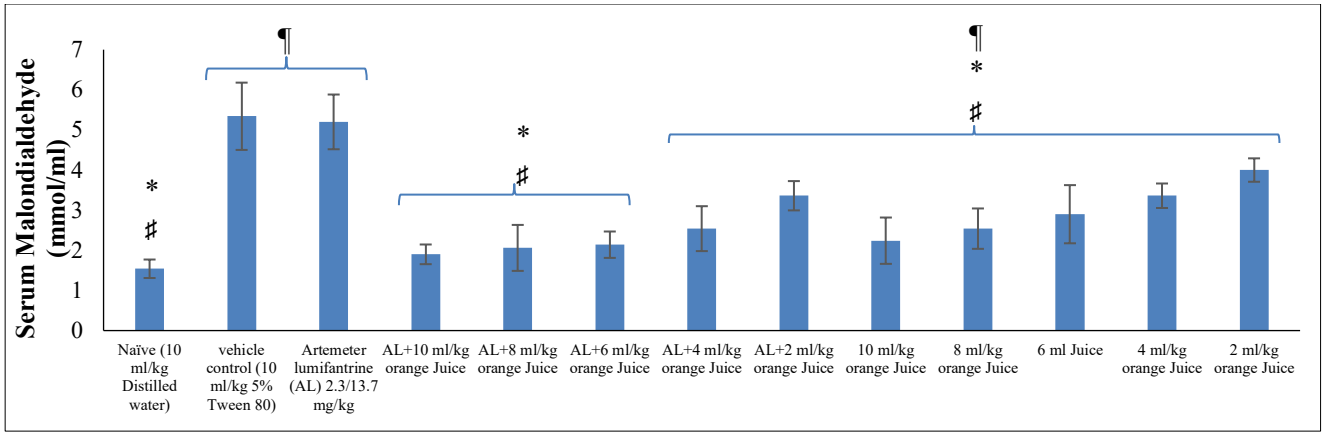

Figure 8: Effect of artemether lumefantrine combination with orange juice on lipid peroxidation.

* P<0.05 compared to vehicle control; ♯ p<0.05 compared to artemether lumefantrine alone; ¶ p<0.05 compared to naïve control.

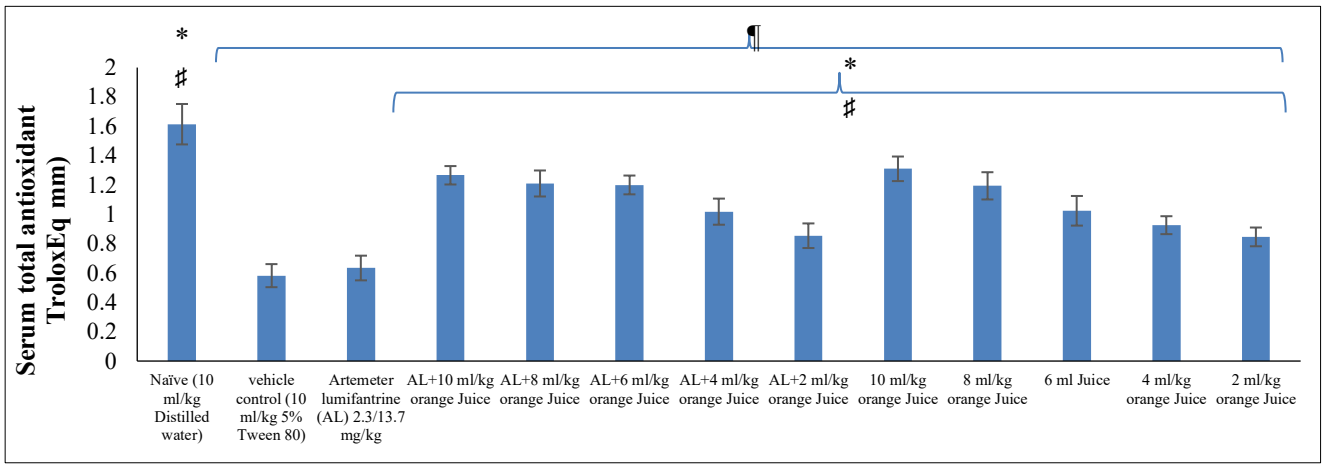

Figure 9: Effect of artemether lumefantrine combination with orange juice on serum total antioxidant.

* P<0.05 compared to vehicle control; ♯ p<0.05 compared to artemether lumefantrine alone; ¶ p<0.05 compared to naïve control.

DISCUSSION

This study assessed the dose and time-dependent effect of orange juice on the antimalarial efficacy of artemether-lumefantrine, as well as its effect on the haematological, and antioxidant parameters.

The findings from this study align with that of previous studies on how malaria and artemisinin-based therapies influence oxidative stress markers. Specifically, a marked increase in lipid peroxidation was noted, as evidenced by elevated malondialdehyde (MDA) levels, which is a common indicator of oxidative stress. Concurrently, there was a noticeable reduction in key antioxidant defenses, including catalase, superoxide dismutase, reduced glutathione, and total antioxidant capacity in both the malaria control and drug-treated groups. Elevated oxidative stress in malaria patients has been well-documented, with increased MDA levels serving as a key biomarker.[14] Similarly, research by Nsiah et al. reported higher oxidative stress in children with severe malaria, demonstrated by raised MDA and decreased ascorbate and hemoglobin concentrations.[15] Atiku et al also found pronounced oxidative stress in individuals with uncomplicated sickle cell disease co-infected with *Plasmodium falciparum*.[16] Other studies have shown that rising parasitemia levels are inversely related to total antioxidant capacity in children with malaria.[17]

Emerging literature supports the use of natural antioxidant agents, such as those derived from plants, fungi, vitamins, or pharmaceutical sources, to potentially enhance host antioxidant defenses and immune responses during malaria infection. These compounds may function by boosting endogenous antioxidant systems or indirectly hindering parasite survival. As a result, supplementation with antioxidants has been suggested to help counteract the oxidative burden associated with both the infection and antimalarial treatments. Nonetheless, according to Isah and Ibrahim, the effectiveness and safety of antioxidant therapy during malaria treatment remain contentious.[18] This is because artemisinin and related antimalarials rely partly on oxidative mechanisms to eliminate parasites, which could be undermined by concurrent antioxidant administration.[18]

Treatment with artemether-lumefantrine alone led to a sharp decline in parasitemia, with complete clearance observed by day 3 to 5, reaffirming the drug's known efficacy as previously validated in earlier studies.[19,20] However, when high doses of orange juice were administered alongside artemether-lumefantrine, parasite clearance appeared to be diminished. This suggests a potential interaction at higher juice concentrations. Such a finding mirrors previous observations by Meshnick et al, who demonstrated that antioxidant vitamins like ascorbate and α-tocopherol could reduce the efficacy of artemisinin-based drugs.[21] Likewise, Oreagba and Ashorobi in 2007 found that retinol negatively affected the activity of dihydroartemisinin against *P. yoelii* in animal models.[22] Awodele et al also documented antagonistic effects of vitamin E on artesunate's activity in *Plasmodium berghei*-infected mice.[7]

Conversely, not all studies agree. Some have reported that combining vitamin C with artemisinin-based therapies such as artesunate–amodiaquine did not significantly impair parasite clearance, with complete eradication achieved by day two.[23] These discrepancies might stem from differences in dosing. In the current investigation, higher doses of orange juice appeared to inhibit drug efficacy, while lower doses seemed to enhance the therapeutic effect and reduce associated oxidative stress. This supports the idea that interactions between combined agents can vary based on the dose ratio. Depending on the combination and dosage, two compounds can exhibit either synergistic or antagonistic effects.[24] Therefore, treatment success in combination therapy may not solely rely on the individual properties of each component but on identifying an optimal dose combination. When two agents are administered together at a defined ratio, the resulting effect can be considered unique and distinct from that of either compound alone.[25] Determining appropriate dose pairs in such combination regimens is essential to optimize efficacy while minimizing toxicity.[26]

An increase in parasitemia observed in the group treated with orange juice alone might be explained by the antioxidant properties of its constituents, especially ascorbic acid. This observation was consistent and showed a dose-dependent trend. The antioxidant action may have inadvertently favoured parasite survival by neutralizing reactive oxygen species, to which malaria parasites are particularly vulnerable.[27] Moreover, earlier studies suggest that at high concentrations, ascorbic acid may suppress parasitemia progression in infected mice.[6] This is in line with our findings, where orange juice at elevated doses independently reduced the rate of *Plasmodium* multiplication when compared to untreated controls.

Limitations

While this study provides valuable insights into the dose and time-dependent interactions between OJ and AL, it has some limitations. The use of *Plasmodium berghei*-infected mice may not fully recapitulate human *P. falciparum* malaria pathophysiology, drug metabolism, or immune responses; hence, extrapolating these findings to human clinical contexts requires caution. Freshly extracted OJ was used without quantifying potential batch-to-batch variability in ascorbic acid or other phytochemicals. Commercial OJ formulations (e.g., pasteurized, fortified) may exhibit different interaction profiles due to processing effects on bioactive compounds. Plasma concentrations of artemether, lumefantrine, or ascorbic acid were not measured. Thus, the mechanistic basis of interactions such as altered drug absorption, metabolism, or tissue distribution remains speculative. Findings are specific to AL. Interactions with other artemisinin-based combination therapies (ACTs) or non-ACT antimalarials may differ.

CONCLUSION

Orange juice showed both antagonistic and beneficial interaction with AL. At higher doses, antagonistic effect was recorded while at lower dose combination with artemether lumifantrine beneficial effect were seen. Combination of orange juice with artemether lumefantrine also has a beneficial effect in reducing both parasite and AL-induced oxidative stress.

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