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Original Research Article

Anti-plasmodial activity of artemether-lumefantrine-tinidazole on plasmodium falciparum infected humans

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ABSTRACT

Background: The emergence of multi-drug resistant strains of *Plasmodium falciparum* has intensified the search for effective compounds. This study aimed to evaluates the combined effects of tinidazole and artemether/lumefantrine on human participants infected with *P. falciparum*.
Methods: The study involved 25 non-infected adults as controls and 75 infected adults, divided into three groups of 25. The groups were treated orally with 1g of Tinidazole (T) twice daily for 3 days, an 8-hourly initial dose followed by 12-hourly 80mg/480mg doses of Artemether/Lumefantrine (AL) for 3 days, and a combination of 1g tinidazole and artemether/lumefantrine (80mg/480mg) for 3 days. Venous blood samples were taken on days 0, 4, and 14 to evaluate renal function, parasitemia, hematological parameters, lipid profiles, and antioxidant measures (catalase, SOD, glutathione peroxidase, and malondialdehyde).
Results: The percentage recovery with AL, T, and the combination of T and AL was 88%, 92%, and 100%, respectively. Malaria patients exhibited significantly lower levels of red blood cells, hemoglobin, packed cell volume, low-density lipoprotein, high-density lipoprotein, total cholesterol, catalase, superoxide dismutase, and glutathione peroxidase, and higher levels of white blood cells, triglycerides, and malondialdehyde compared to the control group. Post-treatment, there was a modest reversal on day 4 and a significant return to normal ranges by day 14. Malaria infection did not affect urea or creatinine levels.
Conclusions: Tinidazole shows potential as a treatment for *P. falciparum*, with enhanced efficacy when combined with artemether/lumefantrine.

Keywords: Anti-plasmodial activity, Artemether-lumefantrine, Hematological parameters, Malaria treatment, *Plasmodium falciparum*, Tinidazole

INTRODUCTION

Malaria remains one of the most significant public health challenges worldwide, particularly in sub-Saharan Africa, where it accounts for substantial morbidity and mortality. *Plasmodium falciparum*, the most virulent of the malaria parasites, is responsible for the majority of severe and fatal cases.[1] According to the World Health Organization's Global Malaria Report 2021, there were about 241 million cases of malaria worldwide in 2020, with an estimated 627,000 deaths. Sub-Saharan Africa bears the brunt, accounting for about 95% of all malaria cases and 96% of all deaths.[2] The emergence of drug-resistant strains of *P. falciparum* necessitates the continual development and evaluation of new therapeutic combinations to ensure effective treatment and control of malaria. Despite global efforts, including those by the World Health Organization and the Global Fund, malaria's impact persists and worsens annually due to drug resistance, limited treatment

accessibility, disease resurgence, and insufficient preventive measures such as insecticide-treated bed nets.[3-6] However, there has been a decline in malaria morbidity and mortality based on recent publications and reports by World Health Organization.[7-10]

The quest for novel antimalarial treatments has led researchers to explore medicinal plants and repurposed drugs.[11,12] Medicinal plants like *Fagara zanthoxyloides* have shown promise in preclinical studies.[13] Additionally, drug repurposing, exemplified by tinidazole and artemether/lumefantrine combinations, offers potential advantages in efficacy and resistance mitigation.

Artemether-Lumefantrine (AL) is currently one of the most widely used artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated *P. falciparum* malaria. Artemether, a derivative of artemisinin, rapidly reduces parasite biomass, while Lumefantrine provides a longer-lasting effect, clearing the remaining parasites and reducing the risk of recrudescence. Despite the efficacy of AL, increasing resistance has raised concerns about its long-term effectiveness.

To address these challenges, alternative or complementary therapies such as tinidazole, initially developed for other parasitic infections, are being explored. Tinidazole, a nitroimidazole derivative, has been primarily used for treating protozoal and anaerobic bacterial infections. Its anti-plasmodial activity has been observed in preliminary studies, suggesting potential utility in combination therapies for malaria. Tinidazole has a safer profile and longer plasma half-life than metronidazole.

Recent research has shown the effectiveness of tinidazole in combination with artemether/lumefantrine in treating P. berghei-infected mice. This study explores the anti-plasmodial activity of a novel combination therapy involving artemether-lumefantrine and tinidazole (ALT), assessing its efficacy in treating *P. falciparum* infections in humans.

The objective of this research was to evaluate the therapeutic efficacy and safety of the ALT combination in patients infected with *P. falciparum*. By investigating the synergistic effects of these drugs, we aim to determine the chemotherapeutic dose required to achieve parasite elimination, evaluate the lipid profile of the treated subjects, and assess antioxidant parameters. The findings of this study could have significant implications for malaria treatment protocols and contribute to the global fight against this pervasive disease.

METHODS

Study setting and population

This study was conducted from November 4th, 2021, to March 15th, 2022, in the Ovom area of Yenagoa City, Bayelsa State, Nigeria, following the World Health Organization's revised protocol for malaria drug therapeutic efficacy studies. The sample size was calculated using a single population proportion formula with a 1% margin of error, 99% confidence interval, 5% treatment failure, and 10% dropout rate. To increase statistical power, 15 additional patients per group were enrolled.

Participants

A total of 100 adult male and female residents of the Ovom area participated in this study. Among them, 75 outpatients presented at The Nigeria Police Medical Services, Yenagoa, Bayelsa State Command, with signs and symptoms of malaria, while 25 were volunteers without symptoms. All participants underwent malaria parasite testing. Eligibility for the clinical trial required a positive malaria parasite test, informed consent, and counseling about the procedure and drugs to be administered. Of the participants, 75 tested positive for malaria and were divided into three treatment groups of 25 each, and 25 malaria-negative participants served as the control group.

Experimental design

Participants were randomly assigned to four groups: 1) Control group (25 participants) with no malaria and no treatment; 2) Tinidazole group (25 participants) treated with 1g of tinidazole twice daily for 3 days; 3) Artemether-Lumefantrine (A/L) group (25 participants) treated with A/L twice daily for 3 days; 4) Combination therapy group (25 participants) treated with 1g of tinidazole and A/L twice daily for 3 days.

Drug administration

Tinidazole, the primary drug repurposed in this study, was administered orally to those who tested positive for malaria parasites, twice daily (morning and evening) for three days. Another group received a combination of tinidazole and A/L. The A/L treatment typically involved an initial dose of 80 mg/480 mg, followed by the same dose twice daily for the next two days. A third group received only A/L (80 mg/480 mg twice daily) for three days.

Assessment of drug efficacy

After completing the treatment course, outpatients returned for a review. Blood samples were taken to examine the malaria parasite load and assess the drug(s) efficacy. The equipment used included a centrifuge BL-110, test tubes, spectrophotometer S23A, and a microscope. Biochemical kits for HDL, LDL, triglycerides, and total protein were sourced from Randox Laboratories LTD, London. Antioxidant assay reagents included TCA, TBA, pyrogallol, HCl, TritonX-100, H_2O_2, H_2SO_4, and EDTA.

Determination of curative activity

The Ryley and Peters method was used to assess the curative activity threshold.[14] Participants were divided into four groups of 25. Group 1 (control) had no malaria and received no treatment. Group 2 received A/L (80/480 mg/kg) for 3 days. Group 3 received tinidazole (1000 mg) twice daily for 3 days. Group 4 received a combination of A/L and tinidazole (80/480 mg + 1000 mg) for 3 days. Clinical symptoms and parasite-positive blood films confirmed malaria infection. The percent recovery was determined using the formula:

$$Percent\ Recovery = \frac{n_o}{N_w} \times 100 \quad (1)$$

Where; n_o is the number of outpatient whom have recovered in a cluster and N_w is the total number of outpatients within the group.

Antioxidant and lipid profile determination

Blood samples (5mL) were collected from the antecubital vein into plain tubes, allowed to clot for 30 minutes, and centrifuged for 10 minutes at 3000 rpm. Serum was collected for antioxidant (catalase, glutathione peroxidase, superoxide dismutase, and malondialdehyde) and lipid profile (HDL, LDL, triglycerides, total cholesterol) analyses.

Lipid peroxidation determination

Lipid peroxidation was measured using malondialdehyde (MDA) production, which forms a pink MDA-TBA complex measured spectrophotometrically at 530 nm. The reaction involved 0.5 ml plasma mixed with 0.5 ml of 20% TCA and 1 ml of 0.67% TBA, heated at 100°C for 15 minutes, then centrifuged at 3000 rpm for 15 minutes. Absorbance was measured at 532 nm.

Catalase activity determination

Catalase activity was measured using the Claiborne (1995) method, involving the breakdown of hydrogen peroxide in the presence of the enzyme. The reaction mixture contained potassium phosphate buffer (pH 7.4), H_2O_2, and plasma. enzyme activity was monitored spectrophotometrically at 240 nm. The catalase activity is obtained using the formula:

$$K = \frac{2.303}{T} \times \log\left(\frac{A1}{A2}\right) \quad (2)$$

where K is the rate of reaction, T is the time interval (mins), $A1$ is the absorbance at time zero, and $A2$ is the absorbance at 60 seconds interval.

Superoxide dismutase determination

Superoxide dismutase (SOD) activity was determined using the Marklund and Marklund method, based on the inhibition of pyrogallol autoxidation.[15] Plasma mixed with Tris-HCl buffer (pH 8.5) and pyrogallol was monitored for absorbance changes at 420 nm over three minutes. The unit of S.O.D per m.L of specimen is obtained using the formula:

$$U_{S.O.D} = \frac{[(A - B) \times 100]}{[A \times 50]} \quad (3)$$

where $U_{S.O.D}$ is the unit of S.O.D per m.l of specimen, A is the variation in absorbance over one minute amid the control and the test specimen, and B is that difference.

Glutathione peroxidase determination

Glutathione peroxidase (GPx) activity was measured using a coupled enzyme assay with glutathione reductase. The reaction mixture included potassium phosphate buffer (pH 7.0), EDTA, sodium azide, reduced glutathione, GR, NADPH, and plasma. The decrease in absorbance at 340 nm was monitored over five minutes at 25°C. The enzyme activity is calculated using the formula:

$$\text{Enzyme Activity} = \frac{\Delta A \times \text{Total Volume} \times \text{Dilution}}{\text{Volume of homogenate} \times 6.22 \times \text{Time}} \quad (4)$$

where 6.22 id the extinction coefficient of NADPH at 340nm and A is the change in absorbance per minute

Total cholesterol determination

Total cholesterol was measured using a biochemical assay. Test tubes containing specimen, standard, and blank were mixed with reagent 1 and incubated at 37°C for 5 minutes. Absorbance was measured at 500 nm.

Triglycerides determination

Triglycerides were measured using a similar biochemical assay. Test tubes containing specimen, standard, and blank were mixed with reagent 1, incubated at 37°C for 5 minutes, and absorbance was measured at 500 nm.

High-density lipoprotein (HDL) determination

HDL cholesterol was measured using the CHOD-PAP method. Specimen and standard solutions were mixed with HDL reagent, incubated, centrifuged, and absorbance was measured at 500 nm.

Low-density lipoprotein (LDL) determination

LDL cholesterol was measured using the CHOD-PAP method. Specimen and standard solutions were mixed with

reagent A, incubated, centrifuged, and absorbance was measured at 500 nm.

By adhering to these methods, this study aims to evaluate the therapeutic efficacy and safety of the artemether-lumefantrine-tinidazole combination in treating *P. falciparum* infections in humans.

RESULTS

The results presented in Table 1 provide valuable insights into the curative activity of tinidazole, artemether/lumefantrine, and their combination in the treatment of malaria-infected outpatients. The significant recovery rates observed on both day 4 and day 14 underscore the effectiveness of these treatment regimens in managing *Plasmodium falciparum* infections.

Table 1: Curative activity of tinidazole, artemether/lumefanthrine and tinidzole + artemether/lumefanthrine in malaria infected outpatients.

	NIP (Day 0)	NRP (Day 4)	NRP (Day 14)	% recovery (Day 4)	% recovery (Day 14)
NC	0	0	0	-	-
AL	25	20[a]	22[a]	80[a]	88[a]
T	25	20[a]	23[a]	80[a]	92[b]
T/AL	25	23[a]	25[b]	92[b]	100[b]

NC: Normal Control; AL: Artemether-Lumefanthrine; T: Tinidazole; T/AL: Tinidazole +AL; NIP: Number of infected persons; NRP: Number of recovery persons

On day 4, the data indicate a significant recovery level in all treatment groups, with artemether/lumefantrine and tinidazole showing a recovery rate of 80% while the combination of tinidazole and artemether/lumefantrine achieved a remarkable 92% recovery. These results demonstrate the rapid action of both tinidazole and artemether/lumefantrine in alleviating the symptoms of malaria and reducing parasitemia. The 100% recovery in the combination group suggests a potential synergistic effect, where the two medications work together to enhance the overall therapeutic outcome (Table 1).

By day 14, all treatment groups exhibited further improvement in recovery rates, with artemether/lumefantrine at 88%, tinidazole at 92%, and the combination group remaining at 100%. The continued increase in recovery rates from day 4 to day 14 indicates that these treatment regimens not only provide rapid relief but also contribute to sustained recovery and eradication of the malaria infection. The increase in recovery percentages suggests that these treatments effectively address the residual effects of the infection and contribute to the complete resolution of symptoms and parasitemia (Table 1).

The data presented in Table 2 illustrate the significant effects of tinidazole, artemether/lumefantrine, and their combination on mean parasitemia in patients infected with *Plasmodium falciparum*. The initial results on day 0 revealed markedly higher levels of parasitemia in the treatment groups compared to the normal control group, highlighting the severity of malaria infection in these patients. The significant differences ($p<0.05$) observed indicate the effectiveness of the treatments in addressing the infection.

Table 2: Mean percentage paracitemia.

	Day 0	Day 4	Day 14	% inhibition (Day 4)	% Inhibition (Day 14)
NC	0	0	0	-	-
AL	57.70±3.6[a]	15.6±4.32[a]	11.0±2.44[a]	72.96±3.12[a]	81.79±2.82[a]
T	59.16±34[a]	14.01±2.44[a]	12.64±5.4[a]	76.32±3.4[a]	78.63±2.16[a]
T/AL	60.75±2.8[a]	7.22±3.63[b]	4.18±2.25[b]	89.22±3.2[b]	93.12±3.2[b]

NC: Normal Control; AL: Artemether Lumefanthrine; T: Tinidazole; T/AL: Tinidazole +AL

By day 4, significant reductions in mean parasitemia were recorded for all treatment groups: artemether/lumefantrine (72.96±3.12), tinidazole (76.32 ± 3.4), and the combination therapy (89.22±3.2). The reduction in parasitemia indicates that both tinidazole and artemether/lumefantrine have potent antimalarial activities, leading to rapid clearance of the parasite from the bloodstream. The combination therapy also showed a notable reduction, although slightly less than the other two groups, which may suggest some variability in response or differences in pharmacokinetics when the two drugs are administered together (Table 2).

By day 14, all treatment groups demonstrated improved mean parasitemia levels compared to day 0, with values of 81.79±2.82 for artemether/lumefantrine, 78.63±2.16 for tinidazole, and 93.12±3.2 for the combination therapy. While these values indicate some remaining parasitemia, the overall improvement underscores the effectiveness of these treatments in achieving long-term reductions in parasite load (Table 2).

The findings presented in Table 3 reveal critical insights into the hematological effects of tinidazole, artemether/lumefantrine, and their combination in malaria-infected patients.

At the onset of treatment (day 0), the malaria-infected patients exhibited significantly decreased levels of red blood cell (RBC) count, hemoglobin (Hb) concentration, and packed cell volume (PCV) across all treatment groups

(artemether/lumefantrine, tinidazole, and the combination) compared to the normal control group. The significant reduction in these parameters indicates the severity of anemia in the patient population at baseline, which is a common complication of malaria. Conversely, the significant increase in white blood cell (WBC) count in the treatment groups suggests an immune response to the malaria infection (Table 3).

Table 3: Effects of tinidazole, artemether/lumefantrine and tinidazole+artemether/lumefantrine on hematological parameters of malaria infected patients.

		RBC (x 10^6/µl)	WBC (x 10^3/µl)	PCV (%)	Hb (g/dl)
Day 0	NC	5.91 ± 2.62^a	4.14 ± 3.51^a	29.88±2.31^a	16.12±1.46^a
	AL	4.52 ± 4.11^b	6.32 ± 2.16^b	22.18±2.71^b	11.62±3.20^b
	T	4.92±2.8^b	6.16±2.61^b	22.45±2.23^b	10.88±22^b
	T/AL	4.6±2.86^b	6.12±2.61^b	21.76±1.22^b	12.83±3.51^b
Day 4	NC	5.60 ± 3.40^a	4.13 ± 2.8^a	29.68 ± 41 ^{a}	16.22±1.89^a
	AL	4.82 ± 3.61^b	6.06 ± 2.14^b	23.12±2.42^b	11.92±2.34^b
	T	5.12±2.23^a	6.09±3.02b ^{b}	23.33±2.26b^b	11.38±3.42^b
	T/AL	5.21±2.86^a	5.96±3.28b^b	23.76±1.2^b	13.03±2.98^b
Day 14	NC	5.44 ± 1.8^a	4.31 ± 2.32^a	29.82 ± 1.6^a	16.62± 3.8^a
	AL	4.89 ± 2.2^a	4.35 ± 1.71^a	29.65±1.8^a	14.9±3.8^a
	T	5.22±3.40^a	4.44±2.16^a	29.34±3.12^a	15.22±2.83^a
	T/AL	5.48±2.14^a	4.14±3.08^a	30.86±3.32^a	16.48±1.84^a

RBC: Red blood cells; WBC: white blood cell; PCV: Packed cell volume; Hb: Hemoglobin

Table 4: Effects of tinidazole, artemether/lumefantrine and tinidazole+artemether/lumefantrine on plasma antioxidant parameters of malaria infected patients.

		Cat (U/ml)	SOD (U/ml)	GPx (U/L)	MDA (µmol/l)
Day 0	NC	9980.42 ±92.2^a	252.69 ± 15.5^a	9088.44± 26.1^a	6.26±0.11^a
	AL	9184.60 ± 97.5^b	142.23 ± 8.7^b	5008.74±38.4^b	11.28±3.2^b
	T	9354.44±288^c	152.66±14.1^c	4471±16.9^c	11.10±1.6^b
	T/AL	9098.45±98.4^b	148.68±8.5^c	4921.32±28.4^b	10.54±2.01^b
Day 4	NC	9700.11±86.92^a	254.87 ± 23.62^a	9091.22 ± 44.3^a	6.25±0.13^a
	AL	9214.44 ± 90.5^b	146.84 ±8.45^b	5026.88±62.8^b	11.32±2.8^b
	T	9224.23±488^b	156.66±4.18^b	4486±56.1^b	9.14±0.36^c
	T/AL	9122.68±96.4^b	151.34±7.23^b	4938.65±42.1^b	9.00±4.16^c
Day 14	NC	9716.44 ±94.2^a	256.23± 12.51^a	9098.11± 96.13^a	6.12±0.22^a
	AL	9742.12 ± 92.6^a	249.34 ± 16.14^a	9060.44±121.44^a	5.94±2.1^a
	T	9989.65±22.9^a	257.12±78.72^a	9054.33±16.45^a	5.91±1.22^a
	T/AL	9924.22±86.7^a	260.01±14.22^a	9156±184.42^a	5.02±0.96^a

CAT: Catalase; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde

By day 4, the results showed an insignificant increase in RBC count, Hb concentration, and PCV across all treatment groups compared to day 0. The lack of statistically significant changes may indicate that the treatments, while beginning to exert their effects, have not yet fully restored the hematological parameters. Additionally, the insignificant decrease in WBC count at this time point suggests that the acute inflammatory response may still be present, although it appears to be starting to normalize (Table 3).

The most notable findings occurred by day 14, where significant increases in RBC count, Hb concentration, and PCV were observed in all treatment groups compared to day 0. This improvement indicates a positive response to the treatments and reflects the recovery from malaria-induced anemia. The rise in these parameters is critical for the restoration of oxygen-carrying capacity and overall patient health, as anemia can lead to fatigue, weakness, and increased morbidity in malaria patients (Table 3).

Furthermore, the significant decrease in WBC count observed at day 14 suggests a normalization of the immune response as the infection is being effectively managed. The reduction in WBC count may indicate a resolution of the inflammatory process associated with malaria, signifying that the body is beginning to regain homeostasis following successful treatment (Table 3).

The data presented in Table 4 provide valuable insights into the effects of tinidazole, artemether/lumefantrine, and their combination on plasma antioxidant parameters in malaria-infected patients. The findings indicate significant alterations in antioxidant enzyme activities and lipid

peroxidation levels throughout the treatment period, reflecting the impact of these therapies on oxidative stress associated with *Plasmodium falciparum* infection.

At baseline (day 0), the results revealed a significant decrease ($p<0.05$) in the activities of plasma antioxidant enzymes, including catalase, superoxide dismutase (SOD), and glutathione peroxidase (Gpx), in the treatment groups (artemether/lumefantrine, tinidazole, and tinidazole + artemether/lumefantrine) compared to the normal control group. Additionally, the significant increase in plasma malondialdehyde (MDA) levels observed at day 0 further supports the presence of oxidative damage in these patients (Table 4).

On day 4, the results showed an insignificant increase in catalase, SOD, and Gpx activities, as well as an insignificant decrease in MDA levels across all treated groups compared to day 0. While these changes did not reach statistical significance, the upward trends in antioxidant enzyme activities indicate a possible early response to treatment. The insignificant decrease in MDA levels suggests a trend toward reduced oxidative stress; however, it may take longer for the treatments to significantly enhance the antioxidant defense system and lower lipid peroxidation levels (Table 4).

By day 14, there was a significant increase in plasma catalase, SOD, and Gpx activities, along with a significant decrease in plasma MDA levels in the treatment groups compared to day 0. These findings indicate a restoration of the antioxidant defense mechanisms, suggesting that the treatments were effective in reducing oxidative stress associated with *Plasmodium falciparum* infection (Table 4).

The data presented in Table 5 highlight the effects of tinidazole, artemether/lumefantrine, and their combination on the plasma lipid profile of malaria-infected patients. The findings indicate significant alterations in lipid levels throughout the treatment duration, revealing the impact of malaria and the therapeutic interventions on lipid metabolism.

At baseline (day 0), patients infected with *Plasmodium falciparum* exhibited significant decreases in plasma low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol levels, alongside an increase in triglyceride levels compared to the normal control group. The reduction in LDL, HDL, and total cholesterol levels suggests that malaria infection may disrupt normal lipid metabolism, potentially due to inflammation, increased catabolism of lipids, or changes in liver function. Conversely, the significant elevation in triglyceride levels is consistent with the metabolic disturbances observed during malaria infection (Table 5).

On day 4, the results indicated an insignificant increase in LDL, HDL, and total cholesterol levels, alongside a significant decrease in triglyceride levels across all treatment groups compared to day 0. Although the changes in LDL, HDL, and total cholesterol were not statistically significant, the upward trends are indicative of a positive response to treatment (Table 5).

Table 5: Effects of tinidazole, artemether/lumefantrine and tinidazole+artemether/lumefantrine on plasma lipid profile of malaria infected patients.

		HDL (mg/dl)	LDL (mg/dl)	Trig. (mg/dl)	Total Chol. (mg/dl)
Day 0	NC	58.12±4.1[a]	35.46±1.15[a]	122.89±2.16[a]	154.45±9.81[a]
	AL	31.65±2.13[b]	27.75±1.88[b]	129.42±9.91[b]	94.33±2.21[b]
	T	29.44±3.02[b]	28.32±2.13[b]	132.3.7±7.93[b]	94.58±8.22[b]
	T/AL	30.51±2.31[b]	27.66±3.81[b]	131.43±10.22[b]	93.64±98.15[b]
Day 4	NC	58.65±6.67[a]	34.88±4.07[a]	121.78 ±2.88[a]	154.89± 3.64[a]
	AL	33.62±4.33[b]	28.66±4.36[b]	127.06±3.11[b]	96.88±3.74[b]
	T	31.38±4.01[b]	29.97±3.11[b]	128.34±4.22[b]	97.44±1.48[b]
	T/AL	33.89±4.23[b]	29.74±5.32[b]	128.43±4.33[b]	96.88±4.38[b]
Day 14	NC	59.03±5.62[a]	34.44±3.25[a]	121.66± 3.75[a]	155.44±6.25[a]
	AL	51.66±2.24[a]	31.56±4.225[a]	120.43±4.16[a]	154.55±2.89[a]
	T	54.54±2.33[a]	30.42±1.9[a]	120.77±6.2[a]	154.33±8.3[a]
	T/AL	52.11±4.14[a]	33.51±4.42[a]	120.45±3.16[a]	155.98±6.44[a]

HDL: High density lipoprotein; LDL: Low density lipoprotein; Trig.: Triglycerides; Total Chol: Total Cholesterol

By day 14, significant increases in LDL, HDL, and total cholesterol levels were observed, along with a significant decrease in triglyceride levels in the treatment groups compared to day 0. These findings indicate a restoration of the lipid profile towards normal values, suggesting that the treatments effectively ameliorated the lipid abnormalities caused by malaria infection (Table 5).

DISCUSSION

This study evaluated the efficacy of tinidazole, artemether/lumefantrine, and their combination in reducing parasitemia in patients infected with *Plasmodium falciparum*. The results indicated significant reductions in mean parasitemia levels at both day 4 and day 14 across all

treatment groups, highlighting the effectiveness of these regimens in managing malaria.

On day 4, the parasitemia levels decreased significantly for all treatment groups, with the combination therapy achieving notable results. These findings are consistent with previous studies that have shown the effectiveness of combination therapies in improving treatment outcomes in malaria. For instance, studies by and demonstrated that artemisinin-based combination therapies (ACTs) rapidly reduce parasitemia and have a lower risk of treatment failure compared to monotherapies.[16,17] The sustained efficacy observed in this study, with further reductions in parasitemia noted by day 14, supports the notion that combination therapies can provide both immediate and long-term benefits, as noted by, who highlighted the importance of maintaining low parasitemia levels to prevent complications associated with malaria.[18]

Additionally, the results align with findings from a study by, which reported significant decreases in parasitemia following treatment with ACTs, reinforcing the effectiveness of these therapeutic strategies.[19] The study noted that such reductions are crucial for improving patient outcomes and reducing the overall burden of malaria.

The observed differences in parasitemia reduction among the treatment groups may also reflect the pharmacokinetics of the drugs used. Tinidazole and artemether/lumefantrine have distinct mechanisms of action against malaria, and their combination may enhance overall efficacy. This is particularly relevant in light of the increasing prevalence of drug-resistant *Plasmodium falciparum*, which necessitates the use of effective multi-drug strategies to combat the disease.[2]

Furthermore, the findings from this study underscore the critical need for ongoing monitoring of treatment efficacy and resistance patterns. As drug resistance continues to pose a significant threat to malaria control efforts globally, employing combination therapies like tinidazole with artemether/lumefantrine may provide a viable strategy to combat this challenge effectively. Continued research into the long-term effects and safety profiles of these treatment regimens will be essential to optimize malaria management.

However, this study provides compelling evidence for the effectiveness of tinidazole, artemether/lumefantrine, and their combination in reducing parasitemia in malaria-infected patients. The findings support the broader literature advocating for combination therapies as a cornerstone of malaria treatment strategies. Future studies should focus on exploring the mechanisms of action of these drugs in combination and their potential impacts on resistance development.

CONCLUSION

This study demonstrates the efficacy of tinidazole, artemether/lumefantrine, and their combination in treating *Plasmodium falciparum*-infected patients. The significant recovery rates, reduction in parasitemia, and improvement in hematological parameters observed in all treatment groups underscore the effectiveness of these regimens in managing malaria. The combination therapy showed a potential synergistic effect, achieving a 100% recovery rate by day 14. Additionally, the treatments restored antioxidant defenses, reduced oxidative stress, and normalized lipid profiles. These findings suggest that tinidazole, artemether/lumefantrine, and their combination are valuable treatment options for malaria, offering rapid relief, sustained recovery, and reduction of residual effects. Further research is warranted to explore the long-term benefits and potential applications of these treatments in malaria-endemic regions.

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