Research Article

Potential antimalarial activity of artemether-lumefantrine-doxycycline: A study in mice infected with *Plasmodium berghei*

Udeme Owunari Georgewill[1*], Elias Adikwu[2]

[1]*Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Rivers State, Nigeria*

[2]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria*

Abstract

Objective: Antimalarial drug resistance is one of the greatest challenges toward eradicating malaria. Exploring new combination therapies can overcome resistance challenges. The present study examined the antiplasmodial effect of artemether/lumefantrine/doxycycline (A/L/D) on a mouse model infected with *Plasmodium berghei* (*P. berghei*). **Materials and Methods**: Swiss albino mice (22-25g) intraperitoneally infected with blood containing $1 \times 10^7 P.$ *berghei* were randomly grouped and orally treated with D (2.2 mg/kg), A/L (2.3/13.7 mg/kg) and A/L/D. The negative and the positive controls were treated with normal saline (0.2ml) and chloroquine (CQ) (10mg/kg) daily for 4 days, respectively. After treatment, blood samples were assessed for percentage parasitemia, biochemical parameters. The mice were also observed for mean survival time (MST). **Results and Conclusion:** In the curative, suppressive and prophylactic tests**,** D, A/Land A/L/D decreased percentage parasitemia levels at $p < 0.05$; $p < 0.01$ and $p < 0.001$ respectively when compared to negative control. In the curative test, D, A/L, A/L/D and CQ produced 60.4%, 70.0%, 81.2% and 76.0% parasitemia inhibitions, respectively. MST was prolonged by D, A/L, and A/L/D at $p < 0.05$; $p < 0.01$ and $p < 0.001$, respectively. D, A/L, and A/L/D prevented *P. berghei-* induced alterations in biochemical parameters by increasing   packed cell volume, red blood cells, hemoglobin, and high-density lipoprotein cholesterol and decreasing white blood cells, cholesterol, total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels significantly at ($p < 0.05$) and ($p < 0.01$) and ($p < 0.001$) when compared to negative control. A/L/D produced significant antiplasmodial activity therefore; it may be used clinically for the treatment of malaria.

Keywords: Antiplasmodial, artemether tetracycline, antimalarial, lumefantrine, *Plasmodium berghei*

Introduction

Antimalarial drug resistance has been acknowledged to be one of the greatest challenges to the Roll Back Malaria programme. The situation is highly precarious due to the rising incidence and increasing *Plasmodium* resistance to currently available antimalarial drugs (Yeung et al.*,* 2004). Chloroquine (CQ) resistant *Plasmodium falciparum* malaria now predominates in Southeast Asia, South America and parts of Africa. Resistance to sulphadoxine-pyrimethamine is widespread in Asia and South America and is spreading in Africa and even quinine has

become less effective over time (Khan et al., 2004). The use of combination therapy with artemisinins and partner drugs has been a rational approach to combating drug resistance. Drugs with different mechanisms of action may enhance their respective efficacies and extend their therapeutic "life spans" (White and Olliaro, 1996). Despite combination therapy approach*, Plasmodium* resistance is still a serious challenge (Yeung et al.*,* 2004).

Doxycycline (D) is one of the most active antibiotics against *Plasmodium* in vitro. It belongs to the tetracycline family. The tetracyclines, which have a very wide spectrum of activity are bacteriostatic and inhibit bacterial protein synthesis (Gillard et al.*,* 2015). Among the tetracyclines, D is widely used for malaria prophylaxis and is highly acceptable for long-term therapy, except in pregnant women and children (WHO, 2005). In vitro and in vivo

*Address for Corresponding Author:
Dr. Udeme Owunari Georgewill
Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Rivers State, Nigeria
Email: udgeorgewill@yahoo.com

investigations have shown that D may be an effective antimalarial drug against drug-resistant *Plasmodium* strains (Basco and Bras, 1993). It is used in combination with quinine as an effective standby emergency treatment for malaria associated with *Plasmodium falciparum* (WHO, 2005).Recently, it has shown antimalarial potential against *Plasmodium berghei*-induced cerebral malaria in experimental model by inhibiting brain inflammation, tumour necrosis factor and chemokines expressions (Schmidt et al*.,* 2018).

Artemether/lumefantrine (A/L) is an artemisinin-based combined antimalarial drug approved by the US Food and Drug Administration in 2009 for the treatment of *P. falciparum* malaria. The dual mechanisms of action of A/L provide fast and sustained *Plasmodium* clearance (Stover *etal.,* 2012). It is the most widely used antimalarial drug combination in endemic regions. In 2017, A/L accounted for almost 75% of all purchased and clinically used artemisinin based combination therapies (ACTs) (Nsanzabana, 2019). Artemisinin derivatives rapidly clear parasites through a number of proposed mechanisms such as interference with plasmodial transport proteins, mitochondrial electron transport, and free radicals production (Stover et al., 2012). Lumefantrine is proposed to inhibit β-hematin formation which is an important detoxification pathway for *Plasmodium* parasites (Stover et al*.,* 2012). Despite the success achieved with the use of A/L to combat malaria scourge, the emergence of *Plasmodium* parasite resistance in some endemic countries has become a significant drawback for the fight against malaria (Nsanzabana, 2019). Due to resistance challenge, recently, there is strong advocacy for the rational use of antimalarial drugs with antibiotics. This will afford the synergistic or additive killing of parasites, and thus prevent the emergence of drug resistance (Miller et al., 2006; Alecrim et al., 2006). This study, therefore assessed if there could be improvement in the antimalarial property of A/L when combined with D in a mouse model infected with *P. berghei.*

Materials and Methods

Drugs and Dose selection

Artemether/lumefantrine (A/L) (IPAC Laboratory, India), Chloroquine (CQ) (Evans Medical Nigeria Plc), Doxycycline (D) (Ranbaxy Laboratories Ltd, India) were used. The following doses were used: A/L (2.3/13.7 mg/kg) (Sirima et al*.,* 2016), CQ (10mg/kg) (Somsak et al*.,* 2018), and D (2.2 mg/kg) (Gilliard et al*.,* 2015).

Animals

Adult Swiss albino mice (22–30 g) of both sexes were used. The mice were obtained from the animal unit of the Department of Pharmacology, Faculty of Basic Clinical Sciences, College of Health Sciences, University of Port Harcourt, Rivers State. The mice were housed and fed throughout the study period as per recommended standards. The mice were allowed for 2 weeks to acclimatize to working environment and were handled according to the international animal care and welfare guidelines (ILAR, 2011).

Parasites inoculation

CQ-sensitive *Plasmodium berghei* (*P. berghei*) (ANKA strain) was used for malaria induction in the experimental mice. *P. berghei* was obtained from Malaria Research Laboratory, Centre for Malaria Research and Phytomedicine, University of Port-Harcourt, Rivers State, Nigeria. Mice previously infected with *P. berghei* were used as donor and parasites were kept alive by continuous intraperitoneal (i.p) passage of blood from donor mouse to uninfected mouse weekly. Percentage parasitemia was determined using the formula below.

Protocol for curative test

The curative test was performed as described by Ryley and Peters (1970). Thirty mice were used for the curative study. Twenty five mice randomly grouped into 5 of 5 mice each were inoculated with 1×10^7 *P. berghei*-infected blood (i.p). Three days later, the mice were treated orally as follows: Group 1 (Normal control) (non-parasitized) was treated with normal saline (0.2ml) daily for 4 days.   Group 2 (Negative control) and 3 (Positive control) were treated with normal saline (0.2ml) and CQ (10mg/kg) for 4 days, respectively. Groups 4-6 were treated with D (2.20mg/kg), A/L (2.3/13.7 mg/kg) and A/L/D daily for 4 days, respectively. On the day 5, tail blood samples were collected from the mice and thin smears were prepared on slides and stained with 10% Giemsa solution. The stained slides were examined microscopically with an oil immersion objective of 100× magnification power. The average percentage parasitemia and inhibitions were calculated.

Protocol for suppressive test

The curative test was performed as described by Knight and Peters (1980). Twenty five mice were inoculated with blood containing 1×10^7 *P. berghei* and randomized into five groups of 5 mice each. The mice were treated after 3 hours of inoculation as follows: Group 1 (Negative control) and 2 (Positive control) were orally treated with normal saline (0.2ml) and CQ (10mg/kg) daily for 4 days, respectively. Groups 3-5 were orally treated with D (2.2mg/kg), A/L (2.3/13.7 mg/kg) and A/L/D daily for 4 days, respectively. From the tail, blood samples were collected from the mice; thin blood films were produced and stained with 10% Giemsa. The stained samples were evaluated

microscopically for percentage parasitemia and inhibitions as explained below:

$$\% \text{ Parasitemia} = \frac{\text{Number of parasitized red blood cells (RBC)}}{\text{Total number of RBC count}} \times 100$$

$$\% \text{ Inhibition} = \frac{\text{Parasitemia of negative control (\%)} - \text{Parasitemia of treated group(\%)}}{\text{Parasitemia of negative control (\%)}}$$

Protocol for prophylactic test

Prophylactic test was performed as described by Peters (1965). Twenty five mice were randomized into 5 groups of five mice each and orally treated as follows. Group 1 (NC) and Group 2 (positive control) (PC) were treated with normal saline (0.2 ml) and CQ (10 mg/kg) daily for 4 days respectively. Groups 3 – 5 were treated with D (2.2 mg/kg), A/L (2.3/13.7 mg/kg), and A/L/D for 4 days, respectively. On day 4, the mice were inoculated with blood containing 1×107 *P. berghei* and treatment continued for 4 days. Tail blood samples were collected on day 8 and percentage parasitemia levels were determined as explained above.

Determination of mean survival time

From the time of inoculation with *P. berghei* until death, mortality of each mouse was monitored and recorded. Mean survival time (MST) was determined using the formula below:

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

Evaluation of biochemical parameters

In the curative study, blood samples were collected from mice and evaluated for red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), white blood cells (WBCs), total cholesterol (CHOL), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), using an auto analyzer.

Statistical analysis

Values were expressed as mean ± SEM (standard error of means) of n=5. Values were analyzed by using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. *p* value of less than 0.05, 0.01 and 0.001 was considered significant.

Results

Curative Antiplasmodial test

Treatment with D, A/L, and A/L/D produced significant decreases in percentage parasitemia levels at $p<0.05$, $p<0.01$, and $p<0.001$ respectively when compared to negative control (PU) (Table 1). D, A/L, A/L/D and CQ produced parasitemia inhibitions of 60.4%, 70.0%, 81.2% and 76.0%, respectively. MST was significantly prolonged in mice treated with D, A/L, and A/L/D at $p<0.05$, $p<0.01$ and $p<0.001$, respectively when compared to negative control (Table 1).

Table 1. Curative effect of artemether/umefantrine/doxycycline on *Plasmodium berghei* infected mice

Treatment	%Parasitemia	% Inhibition	MST (Days)
PU	38.1±4.95	0.0	9.02±0.11
CQ	9.14 ±0.20a	76.0	25.3±2.37 b.
D	15.5±0.67b	60.4	16.2±1.57c
A/L	11.3±0.13a	70.4	24.8±3.48b
A/L/D	7.23±0.08a	81.2	31.6±3.21a

Data as mean ± SEM (Standard Error of Mean) n= 5. PU: Negative control, CQ: Chloroquine, D: Doxycycline, A/L: Artemether/lumefantrine, A/L/D: Artemether/lumefantrine/doxycycline, MST: Mean survival time. [a]$p<0.01$ when compared to PU [c] $p<0.01$, [b] $p<0.01$ when compared to PU, [a] $p<0.001$ when compared to PU.

Table 2. Suppressive effect of artemether/lumefantrine/doxycycline on *Plasmodium berghei* infected mice

Treatment	% Parasitemia	% Inhibition	MST(Days)
PU	20.3±1.03	0.0%	9.43±0.45
CQ	3.33±0.32[a]	83.6%	30.4±3.17[b]
D	7.47±0.11[b]	63.2%	22.7±2.78[c]
A/L	4.04±0.05[a]	80.1%	31.0±3.48[b]
A/L/D	1.56±0.02[a]	92.3%	37.6±3.67[a]

Data as mean ± SEM (Standard Error of Mean) n= 5. PU= Negative control, CQ: Chloroquine, D= Doxycycline, A/L=Artemether/lumefantrine, A/L/D= Artemether/lumefantrine/doxycycline, MST= Mean Survival Time. [a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to PU.

Suppressive antiplasmodial test

Significant decreases in percentage parasitemia levels at p<0.05, p<0.01, and p<0.001 were produced by D, A/L, and A/L/D respectively when compared to negative control (Table 2). Parasitemia inhibitions, which represent 63.2 %, 80.1%, 92.3% and 83.6% were produced by D, A/L, A/L/D and CQ, respectively (Table 2). Treatment with D, A/L, and A/L/D significantly prolonged MST at p<0.05, p<0.01, and p<0.001, respectively when compared to negative control (Table 2).

Prophylactic antiplasmodial test

Percentage parasitemia levels were significantly decreased in mice treated with D (p<0.01), A/L (p<0.001), and A/L/D (p<0.001) respectively when compared to negative control (Table 3). D, A/L, and A/L/D produced parasitemia inhibitions of 65.1%, 82.8%, and 93.9%, respectively whereas CQ produced 86.7%, parasitemia inhibition. Significant prolongation of MST occurred in mice treated with D, A/L, and A/L/D at p<0.05, p<0.01, p<0.001, respectively when compared to negative control (Table 3).

Hematological and lipid profile

P. berghei infected mice showed significantly (p<0.001) increased TG, CHOL, LDL-C WBCs with significantly (p<0.001) decreased Hb, PCV, RBCs and HDL-C when compared to normal control (Tables 5 and 6). But treatment with D, A/L, A/L/D significantly decreased TG, CHOL, LDL-C, WBCs levels and significantly increased Hb, PCV, RBCs and HDL-C levels at p<0.05, p<0.01 and p<0.001, respectively when compared to negative control (Tables 4 and 5).

Table 3. Prophylactic effect of artemether/lumefantrine/doxycycline on *Plasmodium berghei* infected mice

Treatment	% Parasitemia	% Inhibition	MST (days)
PU	18.5±1.57	0.0%	9.66±0.61
CQ	2.46±0.01[a]	86.7%	32.0±3.71 [b]
D	6.46±0.35[b]	65.1%	25.5±2.60 [c]
A/L	2.44±0.17[a]	82.8%	32.6±3.38 [b]
A/L/D	1.23±0.03[a]	93.9%	39.2±4.61 [a]

Data as mean ± SEM (Standard Error of Mean) n= 5, PU= Negative control, CQ= Chloroquine, D= Doxycycline, A/L= Artemether/lumefantrine, A/L/D= Artemether/lumefantrine/doxycycline, MST= Mean survival time. [a]p<0.001, [b]p<0.01, [c]p<0.05 when compared to PU.

Table 4. Effect of artemether-lumefantrine-doxycycline on hematologic parameters of *Plasmodium berghei* infected mice

Treatment	RBC (x10^{6})	WBC (cells/L)	PCV (%)	Hb (g/dL)
NC	5.11±0.24	6.61±0.11	54.6±5.00	16.3±0.08
PU	2.41±0.29[a]	13.9±1.06[a]	20.7±3.17[a]	6.63±0.37[a]
CQ	4.00±0.11[b]	9.11±0.09[b]	38.8±3.54[b]	11.9±0.15[b]
D	3.10±0.17[c]	10.27±0.17[c]	26.5±3.76[c]	9.65±0.45[c]
A/L	3.99±0.12[b]	9.42±0.09[b]	38.4±4.49[b]	10.0±0.80[b]
A/L/D	4.91±0.09[d]	6.33±0.21[d]	50.2±4.21[d]	13.9±0.05[d]

Data as mean ± SEM (Standard Error of Mean) n= 5, NC=Normal control, PU= Negative control, CQ= Chloroquine, D= Doxycycline, A/L=Artemether/lumefantrine, A/L/D= Artemether/lumefantrine/doxycycline, MST= Mean Survival Time, RBC= Red blood cell WBC= White blood cell, PCV= Packed cell volume, Hb= Haemoglobin. [a]p<0.001 when compared to NC, [b]p<0.01, [c]p<0.05, [d]p<0.001 when compared to PU.

Table 5. Effect of artemether-lumefantrine-doxycycline on lipid parameters of *Plasmodium berghei* infected mice

Group	TG (mg/dL)	TCHOL (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
NC	76.8±8.03	100.4±14.0	55.9±5.66	29.1±3.11
PU	266.3±15.0[a]	298.4±14.1 [a]	24.6±2.32 [a]	220.1±18. [a]
CQ	169.1±10.6 [b]	190.8±16.4 [b]	39.1±5.72 [b]	117.8±11.6 [b]
D	210.7±11.6 [c]	247.5±13.7 [c]	30.0±3.19 [c]	175.4±15.0 [c]
A/L	179.0±14.0[b]	199.6±14.3 [b]	37.3±3.67[b]	126.5±12.5 [b]
A/L/D	80.9±7.49[d]	117.8±12.5 [d]	49.5±4.39[d]	41.3±10.1[d]

Data as mean ± SEM (Standard Error of Mean) n= 5. NC=Normal control PU=Negative control CQ=Chloroquine, D=Doxycycline, A/L=Artemether/lumefantrine, A/L/D= Artemether/lumefantrine/doxycycline. TG= Tryglycerides, TCHOL=Total cholesterol, HDL-C= High Density Lipoproteins, LDL-C= Low Density Lipoprotein, [a]p<0.001 when compared to NC, [b]p<0.01, [c]p<0.05, [d]p<0.001 when compared to PU.

Discussion

ACTs are the first line treatment for uncomplicated malaria (WHO, 2015). Unfortunately, the emergence of *Plasmodium* parasites resistant to ACTs has been reported in endemic regions (Cui et al. 2015; WHO, 2016). Antimalarial drugs resistance poses one of the greatest threats to malaria control. In Africa, the efficacy of affordable antimalarial drugs is rapidly declining; whike efficacious antimalarial drugs tend to be too expensive. Cost-effective methods are needed to fight against antimalarial drugs resistance. One of the primary solutions to challenges associated with parasite resistance to antimalarial drugs is to explore new combination therapies. In combination therapy, the possibility of the parasites developing resistance simultaneously to two or more combined drugs with different mechanisms of action is extremely low (Bloland et al., 2000). The current study, therefore examined the antiplasmodial activity of A/L/D in *P. berghei* infected mice. *P. berghei* is used in predicting treatment outcomes for antimalarial drug candidates, due to its sensitivity rate, making it an important parasite for antiplasmodial study (Unekwuojo et al., 2011). Studies have shown that four days suppressive and curative tests are effective in the antiplasmodial evaluation of candidate drugs on early and established infections, respectively. Importantly, suppressive and curative tests give vital information on percent parasitemia, inhibition and mean survival time (MST) (Bobasa et al., 2018). In the current study, treatment with A/L/D produced the best curative and suppressive antiplasmodial effects in relation to individual doses of A/L, D and CQ. In the curative study, treatment with D, A/L, A/L/D, and CQ produced 60.4%, 70.0%, 81.2% and 76.8% inhibitions respectively. In the suppressive study, treatment with D, A/L, A/L/D and CQ produced 63.2 %, 80.1%, 92. 3% and 83. 3% inhibitions, respectively. In addition to percentage parasitemia and parasitemia inhibition, this study determined the MST for the mice used for the curative, suppressive and prophylactic tests to further buttress the antiplasmodial activity of A/L/D (Georgewill et al., 2021). Studies have shown that candidate drugs that can appreciably prolong the MST of parasitized animals compared to the negative control may be active against malaria (Oliveira et al., 2009). In the current study, curative, suppressive and prophylactic tests, A/L/D showed the best prolongation of MST than D, A/L, and CQ alone. In the curative study, D, A/L and A/L/D prolonged MST to 16.2±1.57, 24.8±3.84and 31.6±3.21 days respectively. Studies have shown that *Plasmodium* depend on host hemoglobin as a nutrient-source for growth and multiplication. It consumes more than 75% of haemoglobin during its intra-erythrocytic phase and metabolizes heme into hemozoin (Inbaneson and Sundaram 2012). *P.berghei* infected mice are prone to anemia due to erythrocyte destruction, as a consequence of parasite

multiplication or by spleen reticuloendotelial cell action causing the production of phagocytes by the spleen due to abnormal erythrocytes (Nardos and Makonnen, 2017). In the current study, anemia was conspicuous in *P. berghei* treated rats characterized by decreased PCV, Hb, and RBCs with increased WBCs levels. However, *P. berghei*–induced anemia was vividly reduced in mice treated with A/L/D which was characterized by increased PCV, Hb, and RBCs with decreased WBCs levels. Interestingly, the anti-anemic activity of A/L/D was best when compared to individual doses of A/L, D and CQ.

Changes in serum lipid profile related to malaria infection have been reported in some studies. The underlying biological mechanisms for malaria related lipid changes remain unclear, but may be host related, parasite-related or a combination of the two factors (Visser et al., 2013). The present study observed significant alterations in lipid profile in parasitized mice marked by increased CHOL, TG, LDL-C and decreased HDL-C levels. The altered lipid parameters were best restored in A/L/D treated mice marked by elevated HDL-C and decreased CHOL, TG, LDL-C levels. The observed antiplasmodial effect of A/L/D may be due to independent mechanisms of action of constituent drugs. The antiplasmodial mechanisms of D are not well described, but a number of studies have suggested some mechanisms. As suggested by studies, D may inhibit mitochondrial protein synthesis and also decrease the activity of mitochondrial enzyme (dihydroorotate dehydrogenase) involved in pyrimidine synthesis (Prapunwattana et al., 1998). D can also inhibit the syntheses of nucleotides and deoxynucleotides in *P. falciparum* (Yeo et al., 1997). The artemisinins are speculated to interfere with plasmodial mitochondrial electron transport, transport proteins, and the production of free radicals (Stover et al., 2012). Lumefantrine is thought to inhibit β-hematin formation, an important detoxification pathway for *Plasmodium* (Stover et al., 2012).

Conclusion

In this study, A/L/D decreased percentage parasitemia, increased percentage inhibition and prolonged MST in *P. berghei*-infected mice. The results of this study proposed the use of A/L/D for malaria treatment.

References

Alecrim MG, Lacerda MV, Mourao MP, AlecrimWD, Padilha A, Cardoso BS. 2016. Successful treatment of Plasmodium falciparum malaria with a six-dose regimen of artemether-lumefantrineversus quinine-doxycycline in the Western Amazon region of Brazil. American Journal of

Tropical Medicine and Hygiene, 74: 20-5.

Basco LK, Le Bras J. 1993. Activity in vitro of doxycycline against multidrug-resistant Plasmodium falciparum. Transaction of Royal Society of Tropical Medicine and Hygiene, 87:469–470.

Bobasa EM, Alemu BG, Berkessa ST, Gemechu Y, Fufa FG, Cari GZ, Rike WA. 2018. Antimalarial activity of selected Ethiopian medicinal plants in mice. Journal of Pharmacy & Pharmacognosy Research, 6 (1):57-64.

Cui L, Mharakurwa S, Ndiaye D, Rathod PK, Rosenthal PJ. 2015. Antimalarial drug resistance literature review and activities and finding of the ICEMR network. American Journal of Tropical Medicine and Hygiene, 93(3):57–68.

Gaillard T, Madamet M, Pradines B. 2015. Tetracyclines in malaria. Malaria Journal, 14(445):1-10.

Georgewill UO, Harold Melford, Adikwu E, 2021. Antiplasmodial activity of artemether-lumefantrine-tinidazole *on Plasmodium berghei* infected mice. The Pharmaceutical and Chemical Journal, 8(1):107-113.

Inbaneson SJ, Sundaram R. 2012. In vitro antiplasmodial effect of ethanolic extracts of traditional medicinal plant Ocimum species against Plasmodium falciparum. Asian Pacific Journal of Tropical Medicine, 5(2):103–106.

Institute for Laboratory Animal Research (ILAR). Guide for the care and use of laboratory animals. 8th ed ed. Washington DC: National Academic Press; 2011.

Khan MA, Smego RA Jr, Razi ST, Beg MA. 2004. Emerging drug--resistance and guidelines for treatment of malaria. Journal of the College of Physicians and Surgeons of Pakistan, 14:319-24.

Knight DJ, Peters W. 1980. The antimalarial action of N-Benzyl oxydihydrotriazines and the studies on its mode of action. Annals of Tropical Medicine and Parasitology, 74:393-404.

Miller RS, Wongrichanalai C, Buathong N, McDaniel P, Walsh DS, Knirsch C, Ohrt C. 2006. Effective treatment of uncomplicated Plasmodium falciparum malaria with azithromycin-quinine combinations: a randomized, dose-ranging study. American Journal of Tropical Medicine and Hygiene, 74: 401-6.

Nardos A, Makonnen E. 2017. In vivo antiplasmodial activity and toxicological assessment of hydroethanolic crude extract of Ajugaremota. Malaria Journal, 16:25:1-8.

Nsanzabana C. 2019. Resistance to Artemisinin Combination Therapies (ACTs): Do Not Forget the Partner Drug! Tropical Medicine and Infectious Disease, 4(26):1-11.

Oliveira AB, Dolabela MF, Braga FC, Jácome RLRP, Varotti FP, Póvoa MM. 2009. Plant-Derived Antimalarial Agents: New Leads and Efficient Phythomedicines. Part I. Alkaloids. Anais da Academia Brasileira de Ciências, 81:715–40.

Peters W. 1967. Rational methods in the search for antimalarial drugs. Transaction of Royal. Society of Tropical Medicine and Hygiene, 61:400-410.

Prapunwattana P, O'Sullivan WJ, Yuthavong Y. 1988. Depression of *Plasmodium falciparum* dihydroorotate dehydrogenase activity in in vitro culture by tetracycline. Molecular and Biochemical Parasitology, 27:119–24.

Ryley JF, Peters W. 1970. The antimalarial activity of some quinolone esters. Annals of Tropical Medicine and Parasitology, 84:209-222.

Schmidt KE, Kuepper JM, Schumak B, Alferink J, Hofmann A, Hawland SW, Renia L, Limmer A, Specht S. Hoerauf A. 2018. Doxycycline inhibits experimental cerebral malaria by reducing inflammatory immune reactions and tissue-degrading mediators. Public Library of Science One, 13:e0192717.

Sirima SB, Ogutu B, Lusingu JPA. 2016. Comparison of artesunate-mefloquine and artemether-lumefantrine fixed-dose combinations for treatment of uncomplicated Plasmodium falciparum malaria in children younger than 5 years in sub-Saharan Africa: a randomized, multicentre, phase 4 trial. Lancet Infectious Disease, 16(10):1123-1133.

Somsak V, Damkaew A, Onrak P. 2018. Antimalarial Activity of Kaempferol and Its Combination with Chloroquine in Plasmodium berghei infection in mice. Journal of Pathology, 2018;1-7.

Stover KR, King ST, Robinson J. 2012. Artemether-lumefantrine: an option for malaria. Annals of Pharmacotherapy, 46(4):567-77.

Unekwuojo EG, James O, Olubunmi AR. 2011. Suppressive, curative and prophylactic potentials of Morindalucida (Benth) against, erythrocytic stage of mice infected with Chloroquine sensitive Plasmodium berghei. British Journal of Applied Science and Technology, 1(3):131-40.

Visser BJ, Wieten RW, Nagel IM. Grobusch MP. 2013. Serum lipids and lipoproteins in malaria - a systematic review and meta-analysis. Malaria Journal, 12(442):1-16.

White NJ, Olliaro PL, 1996. Strategies for the prevention of antimalarial drug resistance: rationale for combination chemotherapy for malaria. Parasitology Today 12:399–401.

World Health Organization. 2005. International travel and health, World Health Organization, Geneva, Switzerland, p. 132–151.

Yeung S, Pongtavornpinyo W, Hastings IM, Mills AJ, White NJ. 2004. Antimalarial drug resistance, artemisinin based combination therapy, and the contribution of modelling to elucidating policy choices. American Journal of Tropical Medicine and Hygiene, 71(2):179-86.