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In-vivo Antiplasmodial Activity of Sulfadoxine/Pyrimethamine/Doxycycline on *Plasmodium berghei* Infected Mice

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Authors' contributions

This work was carried out in collaboration between both authors. Authors UOG and EA designed the study, performed literature search, managed statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The search for newer antimalarial drug combinations is on the front burner due to rising *Plasmodium* resistance to some currently used antimalarial drugs. This study examined the antiplasmodial activity of sulfadoxine/pyrimethamine/doxycycline (S/P/D) on mice infected with *Plasmodium berghei* (*P. berghei*). Swiss albino mice (25-30 g) inoculated with *P. bergei* ($1x10^7$) were treated with D (2.2 mg/kg), S/P (21.4/10.7 mg/kg), and S/P/D for 4 days. The positive and negative controls were treated with normal saline (0.2 ml) and chloroquine (CQ) (10 mg/kg) for 4 days, respectively. After treatment, blood samples were collected and assessed for parasitemia levels and biochemical parameters. The mice were observed for mean survival time (MST). D, S/P, S/P/D and CQ significantly decreased parasitemia in the curative, prophylactic and suppressive tests at $p<0.05$; $p<0.01$, $p<0.001$ and $p<0.001$, respectively when compared to negative control. In the curative study, 55.9%, 65.1%, and 81.7% parasitemia inhibitions were produced by D, S/P and S/P/D, respectively whereas CQ produced 75.6 % parasitemia inhibition. D, S/P and S/P/D significantly prolonged MST at $p<0.05$, $p<0.01$ and $p<0.001$ respectively when compared to negative control.

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Altered serum biochemical markers in *P. berghei* infected mice were marked by significantly ($p<0.001$) decreased packed cell volume, red blood cells, hemoglobin, high density lipoprotein cholesterol levels with significantly ($p<0.001$) increased cholesterol, white blood cells, total cholesterol, low-density lipoprotein cholesterol and triglyceride levels when compared to control. However, D, S/P and S/P/D significantly restored the aforementioned markers at $p<0.05$, $p<0.01$ and $p<0.001$, respectively when compared to negative control. S/P/D may be used as an antimalarial drug.

Keywords: Antiplasmodial; sulfadoxine/pyrimethamine; doxycycline; combination; mice.

1. INTRODUCTION

Malaria is a major public health threat despite laudable progress on its control in the past decade. The World Health Organization reported 228 million cases of malaria and 405,000 malaria deaths in the year 2018, with the African region having 93% of all cases [1]. The current treatment for malaria requires the use of artemisinin-based combination therapies (ACTs), which consist of artemisinin derivatives with partner drugs. ACTs have decreased malaria-associated mortality in malaria endemic regions [2]. However, this achievement is threatened by decrease in the efficacy of artemisinins, which is marked by decreased parasitic clearance and recrudescence [3]. Resistance to partner drugs has been shown to be facilitated by pre-existing increase in resistance to artemisinin component of the ACTs [2]. Reports showed that challenges associated with *Plasmodium* resistance to artemisinins and partner drugs may be overcome by the incorporation of antibiotics due to promising antimalaria activity displayed by some antibiotics [4,5].

Sulfadoxine/pyrimethamine (S/P) has been used for the treatment of uncomplicated malaria, and as an intermittent preventive treatment for vulnerable populations, infants and pregnant women in malaria endemic regions. S/P is used in combination with artemisinins for malaria treatment in most malaria endemic regions [6] and intermittent preventive treatment for malaria in pregnancy in some African countries [7]. S/P is also used in combination with amodiaquine for malaria chemoprevention [8]. However, there is emergence and wide spread resistant to S/P by *Plasmodium* parasites primarily *P. falciparum* [9]. This resistance has been attributed to point mutations in its target enzymes (dihydropteroate synthase and dihydrofolate reductase) which are involved in folate biosynthesis pathway [10].

Doxycycline (D) belongs to the tetracyclines family of broad-spectrum antibiotics. It is active against protozoa including *Plasmodium*. It inhibits bacterial protein synthesis by binding to proteins in the 30S ribosomal subunit and to ribonucleic acids in the 16S ribosomal RNA [11]. In 1994, after its development, D was approved for malaria prophylaxis by the US Food and Drug Administration. D can be used by travelers to all malaria-endemic areas for malaria prophylaxis [12]. It is a slow-acting blood schizontocidal agent that is highly effective at preventing malaria [13]. In areas with chloroquine and multidrug-resistant *P. falciparum* parasites, D has been used successfully in combination with quinine to treat malaria, and has been proven to be effective and well-tolerated [12]. This study therefore assessed whether a combination of sufadoxine/pyrimethamine with doxycycline (S/P/D) could be effective for malaria treatment using a mouse model infected with *Plasmodium berghei* (*P. berghei*).

2. METHODOLOGY

2.1 Animals, Drugs and Dose Selection

Swiss albino mice (25–30 g) were obtained from the Animal House, of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Nigeria. The mice were housed in plastic cages, acclimated for 2 weeks and had *ad libitum* access to food and water. Doxycycline (D) (Ranbaxy Laboratories Ltd, India), sulfadoxine/ pyrimethamine (S/P) (IPAC Laboratory, India), and chloroquine (CQ) (Evans Medical Nigeria Plc) were used. The doses used are S/P (21.4/10.7 mg/kg) [14], D (2.2 mg/kg) [12] and CQ (10mg/kg) [15].

2.2 Inoculation of Mice with Parasite

CQ sensitive strain of *P. berghei* (NK 65) was obtained from donor mice supplied by the Nigerian Institute of Medical Research Yaba, Lagos. The parasite was maintained by serial

passage of blood from donor mice to non-infected mice within 5–6 days of infection. Donors with a parasitemia level (20–30%) were sacrificed and blood collected by cardiac puncture into heparinized tubes. The blood samples were diluted with normal saline and the experimental mice were treated with blood containing 1×10^7 *P. berghei* intraperitoneally (i.p).

2.3 Antiplasmodial test

2.3.1 Curative test

It was performed as explained by Ryley and Peters [16].Thirty Swiss albino mice were used, twenty five mice were infected i.p with 1×10^7. *P. berghei*. The mice were randomized into 6 groups of n=5 each. The normal control (Non – parasitized) and the positive controls were orally treated with normal saline (0.2 mL) daily for 4 days. The negative control was orally treated with CQ (10 mg/kg) daily for 4 days. Other groups were orally treated with D (2.2 mg/kg), S/P (21.4/10.7 mg/kg) and S/P/D respectively. S/P was administered once whereas D was administered daily for 4 days. On the 5th day, blood samples were obtained from the tail of the mice. Thin blood films were produced on slides and stained with Giemsa stain. The stained slides were viewed using a microscope and percentage parasitemia and percentage inhibitions were calculated using the formula below.

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

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

2.3.2 Prophylactic test

It was carried out as described by Peters [17]. Twenty five Swiss albino mice were randomized into 5 groups of n=5. The positive and negative controls were orally treated with normal saline (0.2 ml) and CQ (10 mg/kg), daily for 4 days, respectively. Other groups were orally treated with D (2.2 mg/kg), S/P (21.4/10.7 mg/kg) and S/P/D respectively. S/P was administered once whereas D was administered daily for 4 days. On the 5th day, the mice were infected i.p with blood containing $1x10^7$ *P. berghei*. After infection, treatment continued for 4 days. On the 8th day, blood samples were obtained from the tail of the mice. The blood samples were processed on slides, stained with Gemsa stain and percentage parasitemia and inhibitions were calculated as shown above.

2.3.3 Suppressive test

It was carried out as reported by Knight and Peters [18]. Twenty-five Swiss albino mice inoculated i.p with blood containing $1x10^7$ *P. berghei* were used. After 72 h, the mice were grouped into 5 of n=5 and were orally treated with D (2.2 mg/kg), S/P (21.4/10.7 mg/kg) and S/P/D, respectively. S/P was administered once whereas D was administered daily for 4 days. The positive and negative controls were orally treated with normal saline (0.2 mL) and CQ (10 mg/kg), respectively for 4 days. On the 5th day, blood samples were obtained from the tail of the mice, stained with Gemsa stain and viewed using a microscope. Thereafter, percentage parasitemia and inhibitions were calculated as shown above.

2.3.4 Evaluation of biochemical markers

Mice in the curative group were anesthetized in a diethyl ether chamber. Blood samples were collected from the heart and evaluated for hemoglobin (Hb), white blood cells (WBCs), red blood cells (RBCs), packed cell volume (PCV), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TCHOL), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) using standard test kits according to manufacturer's specification.

2.3.5 Evaluation of mean survival time

During the study, mortality of each mouse was monitored and recorded. Mean survival time (MST) was calculated as shown below

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

2.4 Statistical Analysis

Values are expressed as mean ± SEM (standard error of mean) of n=5. One-way analysis of variance (ANOVA) and Tukey's *post hoc test* were used for the analysis of data. *Probability (p)* values less than 0.05, 0.01 and 0.001 were considered significant

3. RESULTS

3.1 Curative Effect of Sulfadoxine/ Pyrimethamine/ Doxycycline on *Plasmodium berghei* Infected Mice

Significant decreases in percentage parasitemia at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.001$ were produced by D, S/P, S/P/D and CQ, respectively when compared to negative control. Treatment with D, S/P and S/P/D produced 55.9%, 65.1%, and 81.7% parasitemia inhibitions, respectively whereas CQ produced 75.6% paracetamia inhibition (Table 1). MST was significantly prolonged by D, S/P, S/P/D and CQ at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.001$, respectively when compared to the negative control (Table 1)

3.2 Prophylactic Effect of Sulfadoxine/ Pyrimethamine/ Doxycycline on *Plasmodium berghei* Infected Mice

Parasitaemia levels were significantly decreased by D, S/P, S/P/D and CQ at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.001$, respectively when compared to the negative control. The parasitemia inhibitions produced by D, S/P and S/P/D were 67.1%, 72.8%, and 90.9%, respectively whereas CQ produced 85.0%, parasitemia inhibition (Table 2). Treatment with D, S/P, S/P/D and CQ significantly prolonged MST at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.001$, respectively when compared to the negative control (Table 2).

3.3 Suppressive Effect of Sulfadoxine/ Pyrimethamine/ Doxycycline on *Plasmodium berghei* Infected Mice

Treatment with D, S/P, S/P/D and CQ significantly decreased parasitemia levels at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.001$, respectively when compared to negative control (Table 3). Parasitemia inhibitions produced by D, S/P and S/P/D were 60.7%, 70.6%, and 83.7%, respectively whereas CQ produced 80.0% parasitaemia inhibition (Table 3). MST was significantly prolonged at $p<0.05$, $p<0.01$ $p<0.001$ and $p<0.001$ by D, S/P, S/P/D and CQ, respectively when compared to negative control (Table 3).

3.4 Effect of Sulfadoxine/ Pyrimethamine/ Doxycycline on Hematological and Lipid Profile of *Plasmodium berghei* Infected Mice

P. berghei infected mice showed significant increases ($p<0.001$) in serum WBCs, TG, LDL-C and TCHOL levels with significant ($p<0.001$) decreases in serum RBCs, Hb, HDL-C and PCV levels when compared to the normal control (Tables 4 and 5). However, serum WBCs, TG, LDL-C and TCHOL levels were decreased whereas RBCs, Hb, HDL-C and PCV levels were increased significantly by D, S/P, S/P/D and CQ at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.001$, respectively when compared to the negative control (Tables 4 and 5).

4. DISCUSSION

ACTs are the current treatments for uncomplicated malaria especially for malaria associated with *Plasmodium falciparum* [19]. Unfortunately, emerging *Plasmodium* parasite resistance to ACTs is one of the nagging issues associated with malaria chemotherapy. Parasites have also consistently developed resistance to other classes of antimalarial drugs [20].

The problem is appalling and thus makes the search for newer antimalarial drugs or exploring newer antimalarial drug combinations imperative. This study explored the antiplasmodial activity of sulfadoxine/pyrimethamine/doxycycline (S/P/D) on mice infected with *P. berghei*. Rodent malaria parasites have been widely used as experimental models due to their adaptability to laboratory conditions. *P. berghei* (NK-65) is a lethal rodent malaria parasite experimentally used for the assessments of antimalarial drug candidates. Mice infected with *P. berghei* die within 7–10 days of infection in normal course of infection [21]. The 4-day curative and suppressive tests are standard tests used for the evaluations of antimalarial drug candidates. These tests give vivid information on parasitemia density and percentage inhibition. The *in-vivo* model is used for antimalarial studies because it allows the possible prodrug effect and likely boosting of the immune system in the eradication of an infectious agent [22]. Studies have used CQ as a reference drug in curative, suppressive and prophylactic antiplasmodial evaluation models [23].

Table 1. Curative effect of sulfadoxine/pyrimethamine/doxycycline on *Plasmodium berghei* infected mice

Treatment	%Parasitemia	%Inhibition	MST (Days)
NC	38.10±4.32	0.0	9.00±0.18
CQ	9.29 ±1.54[a]	75.6	29.62±2.43 [a].
D	16.80±1.27[b]	55.9	18.85±1.19[b]
S/P	13.30±1.11[c]	65.1	23.81±3.47 [c]
S/P/D	6.97±0.15[a]	81.7	32.05±3.69 [a]

Values are expressed as ± SEM, n= 5, NC: Negative control, CQ: Chloroquine, D: Doxycycline, S/P: Sulfadoxine/Pyrimethamine,, MST: Mean Survival Time. [a] p<0.001, [b] p<0.05, [c] p<0.01 significant difference when compared to NC. SEM: Standard error of the mean

Table 2. Suppressive effect of sulfadoxine/pyrimethamine/doxycycline on *Plasmodium berghei* infected mice

Treatment	% Parasitemia	% Inhibition	MST(Days)
NC	24.80±2.78	0.0%	9.21±0.56
CQ	4.96±0.01[a]	80.0%	32.92±3.21[a]
D	9.75±0.18[b]	60.7%	22.10±1.53 [b]
S/P	7.29±0.29[c]	70.6%	25.31±2.36 [c]
S/P/D	4.04±0.07[a]	83.7%	34.62±4.10 [a]

Values are expressed as ± SEM, n= 5. NC: Negative control, CQ: Chloroquine, D: Doxycycline, S/P: Sulfadoxine/pyrimethamine, MST: Mean survival time. [a] p<0.001, [b] p<0.05, [c] p<0.01 significant difference when compared to NC, SEM : Standard error of the mean

Table 3. Prophylactic effect of sulfadoxine/pyrimethamine/doxycycline on *Plasmodium berghei* infected mice

Treatment	% Parasitemia	% Inhibition	MST (days)
NC	20.50±2.33	0.0%	9.50±0.67
CQ	4.10±0.26[a]	85.0%	32.41±3.67 [a]
D	6.74±0.37[b]	67.1%	20.73±3.11 [b]
S/P	5.58±0.14[a]	72.8%	27.91±2.07 [c]
S/P/D	0.43±0.08[a]	90.9%	36.02±4.50 [a]

Values are expressed as ± SEM, n= 5. NC: Negative control, CQ: Chloroquine, D: Doxycycline, S/P: Sulfadoxine/pyrimethamine,, MST: Mean Survival Time. [a] p<0.001, [b] p<0.01, [c] p<0.05 significant difference when compared to NC. SEM: Standard error of the mean

Table 4. Effect of sulfadoxine/pyrimethamine/doxycycline on hematological markers of *Plasmodium berghei* infected mice

Treatment	RBCs ($x10^6$)	WBCs (cells/L)	PCV (%)	Hb (g/dL)
C	5.57±0.61	6.68±0.10	65.51±3.00	15.93±2.00
PC	2.01±0.56[a]	14.93±1.24[a]	23.14±2.67[a]	7.16±0.32[a]
CQ	5.01±0.76[b]	8.67±0.33[b]	56.23±5.49[b]	14.91±1.56[b]
D	3.66±0.37[c]	11.12±0.51[c]	37.00±2.10[c]	9.33±0.72[c]
S/P	4.53±0.18[d]	9.00±0.27[d]	45.61±4.43[d]	12.05±0.26[d]
S/P/D	5.20±0.33[b]	6.90±0.53[b]	59.93±5.67[b]	15.24±1.55[d]

Values are expressed as ± SEM, n= 5. C: Normal control PC: Negative control CQ: Chloroquine, D: Doxycycline, S/P: Sulfadoxine/Pyrimethamine, MST: Mean Survival Time, RBCs: Red Blood Cells , WBCs: White Blood Cells, PCV: Packed Cell Volume, Hb: Haemoglobin. [a] p<0.001 significant difference when compared to C, [b] p<0.001, [c] p<0.05, [d] p<0.01 significant difference when compared to PC, SEM :Standard error of the mean

Table 5. Effect of sulfadoxine/pyrimethamine/doxycycline on lipid markers of *Plasmodium berghei* infected mice

Group	TG (mg/dL)	TCHOL (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
C	76.8±6.71	100.9±12.0	65.4±6.71	44.2±4.33
PC	273.0±15.0[a]	352.0±18.9[a]	20.9±2.70[a]	201.1±12.7[a]
CQ	88.8±6.61[b]	131.1±20.7[b]	56.9±5.00[b]	111.9±10.9[b]
D	160.3±12.7[c]	270.0±14.4[c]	33.8±4.76[c]	149.3±13.6[c]
S/P	120.5±11.8[d]	200.7±10.9[d]	42.5±4.58[d]	100.2±12.8[d]
S/P/D	80.6±6.43[b]	122.7±11.0[b]	59.0±4.02[b]	60.3±8.13[b]

Values are expressed as mean ± SEM, n= 5. C: Normal control PC: Negative control CQ: Chloroquine, D: Doxycycline, S/P: Sulfadoxine/Pyrimethamine, TG: Tryglycerides, HDL: High Density Lipoprotein Cholesterol, TCHOL: Total cholesterol, LDL: Low Density Lipoprotein Cholesterol.[a] p<0.001 significant difference when compared to C,[b] p<0.001,[c] p<0.05,[d] p<0.01 significant difference when compared to PC. SEM: Standard error of the mean

In the current study, in the curative and suppressive tests, D, S/P, S/P/D and CQ decreased parasitemia with most decrease produced by S/P/D. S/P/D also produced most percentage inhibition than individual doses of D, S/P and CQ. In the prophylactic test, this study observed that S/P/D produced most reduction in parasitemia and percentage inhibition than individual doses of D, S/P and CQ. One of the challenges associated with malaria treatment is *Plasmodium* parasite recrudescence which causes mortality if untreated [24]. It is therefore suggested that in antiplasmodial studies, follow-up of mice for at least 30 days is imperative to monitor the efficacy of candidate drugs for malaria treatment [25]. Hence, this study observed the mice for mortality which was recorded as MST in days. In the curative, suppressive and prophylactic tests, the current study observed that MST was most prolonged by S/P/D than individual doses of D, S/P and CQ. Studies showed that anemia is one of the primary complications of severe malaria especially in endemic regions [25]. This study observed anemic condition in *P. berghei* infected mice. This was characterized by decreased serum Hb, RBCs, and PCV levels with increased WBCs levels. The observed decreased serum Hb, RBCs, and PCV is an indication of anemia, due to haemolysis. Anemia is a consistent finding in blood protozoan parasites infection. Some earlier investigators have observed a progressive fall in RBCs, PCV and Hb values in *Plasmodium* infections [26]. However, treatment with D, S/P and S/P/D curtailed *P berghei*-induced anemia. This was characterized by increased serum Hb, RBCs, and PCV levels with decreased serum WBCs levels. Anemia was most curtailed by S/P/D when compared to individual doses of D, S/P and CQ. Several studies have observed serum lipid changes during malaria infection in humans. A meta-analysis showed alterations in TG, TCHOL, HDL-C and LDL-C concentrations in malaria compared to both healthy controls and to other febrile diseases [27]. The current study observed altered lipid levels in *P. berghei* infected mice marked by increased TG, TCHOL, and LDL-C and decreased HDL-C levels. Similarly, Georgewill et al. [28] reported altered lipid profile in *P. berghei* infected mice. In this study, treatment with S/P/D restored lipids levels best than individual doses of D, S/P and CQ.

In the current study, S/P/D produced significant antiplasmodial activity which is a function of the individual mechanisms of action of the constituent drugs. The antiplasmodial action of D is not well defined, but it has been speculatively attributed to the inhibition of the syntheses of *Plasmodium* nucleotides, mitochondrial protein, and deoxynucleotides [29,30]. The antiplasmodial action of S/P has been well defined. P, an antifolate drug inhibits dihydrofolate reductase, an essential enzyme in parasite's folic acid pathway. S, an analogue of *p*-aminobenzoic acid competitively inhibits dihydropteroate synthase, which is required for folate biosynthesis [31]. CQ concentrates in parasite food vacuole where it binds free hematin forming CQ-hematin complex and hemoglobin thereby causing parasite death [32].

5. CONCLUSION

Based on the observation in this study, S/P/D produced most antiplasmodial activity than individual doses of D, S/P and CQ. S/P/D also restored hematological and lipid parameters most than individual doses of D, S/P and CQ. S/P/D may be an effective antimalarial drug.

ETHICAL APPROVAL

The experiment was performed according to the guideline on animal handling produced by European Council and the Parliament.

DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization World Malaria Report; 2019. Geneva
2. Ouji M, Augereau JM, Paloque L, Benoit-Vical F. *Plasmodium falciparum* resistance to artemisinin-based combination therapies: A sword of damocles in the path toward malaria elimination. Parasite. 2018; 25:24:1-12.
3. Dondorp AM, Smithuis FM, Woodrow C, Seidlein L von. How to contain artemisinin- and multidrug-resistant falciparum malaria. Trends in Parasitology. 2017;33:353–363.
4. Miller RS, Wongrichanalai C, Buathong N, McDaniel P, Walsh DS, Knirsch C, Ohrt C. Effective treatment of uncomplicated *Plasmodium falciparum* malaria with azithromycin-quinine combinations: a randomized, dose-ranging study. American Journal of Tropical Medicine and Hygiene. 2006;74:401-6.
5. Alecrim MG, Lacerda MV, Mourao MP, Alecrim WD, Padilha A, Cardoso BS. Successful treatment of *Plasmodium falciparum* malaria with a six-dose regimen of artemether-lumefantrine versus quinine-doxycycline in the Western Amazon region of Brazil. American Journal of Tropical Medicine and Hygiene. 2016;74:20-5.
6. WHO. World Malaria Report 2018; WHO: Geneva, Switzerland. 2018;210.
7. Desai M, Hill J, Fernandes S, et al. Prevention of malaria in pregnancy. Lancet Infectious Disease. 2018;18:e119–e132.
8. Coldiron ME, Von Seidlein L, Grais RF. Seasonal malaria chemoprevention: Successes and missed opportunities. Malaria. Journal. 2017;16; 481.
9. Basuki S, Fitriah, Risamasu PM, et al. Origins and spread of novel genetic variants of sulfadoxine–pyrimethamine resistance in *Plasmodium falciparum* isolates in Indonesia. Malaria Journal. 2018;17(475):2-14.
10. Nsanzabana C. Resistance to artemisinin combination therapies (ACTs): do not forget the partner drug! Tropical Medicine and Infectious Disease. 2019;4(26):1-11.
11. Sharma I, Sullivan M, McCutchan TF. The in vitro anti-malarial activity of novel semi synthetic nocathiacin I antibiotics. Antimicrobial Agents Chemotherapy. 2015; 59:3174–9.
12. Gaillard T, Madamet M, Pradines B. Tetracyclines in malaria. Malaria Journal. 2015;14(445):1-10.
13. Tan KR, Magill AJ, Parise ME, Arguin PM. Centers for disease control and prevention. doxycycline for malaria chemoprophylaxis and treatment: report from the CDC expert meeting on malaria chemoprophylaxis. American Journal of Tropical Medicine and Hygiene. 2011;84(4):517-531.
14. Mikomangwa WP, Minzi O, Mutagonda R, *et al.* Effect of sulfadoxine-pyrimethamine doses for prevention of malaria during pregnancy in hypoendemic area in Tanzania. Mal J. 2020;19(160):1-11.
15. Somsak V, Damkaew A, Onrak P. Antimalarial activity of kaempferol and its combination with chloroquine in *Plasmodium berghei* infection in mice. J Path. 2018;1-7.
16. Ryley JF, Peters W. The antimalarial activity of some quinolone esters. Ann Trop Med and Parasit. 1970;84:209-222.
17. Peters W. Rational methods in the search for antimalarial drugs. Transact Royal Soc Trop Med and Hyg. 1967;61:400- 410.
18. Knight DJ, Peters W. The antimalarial action of N-Benzyl oxydihydrotriazines and

the studies on its mode of action. Ann of Trop Med and Parasit. 1980;74:393-404.
19. WHO. World malaria report. Geneva; 2015.
20. Cui L, Mharakurwa S, Ndiaye D, Rathod PK, Rosenthal PJ. Antimalarial drug resistance literature review and activities and finding of the ICEMR network. Am J Trop Med and Hyg. 2015;93(3):57–68.
21. Chandel S, Bagai U, Vashishat N. Antiplasmodial activity of *Xanthium strumarium* against *Plasmodium berghei*-infected BALB/c mice. Parasit Res. 2012;110:1179–83.
22. Waako P, Gumede B, Smith P, Folb P. The in vitro and in vivo antimalarial activity of *Cardiospermum halicacabum* L. and *Momordica foetida* Schumch. Et Thonn. J Ethnopharmacol. 2005;99:137–43.
23. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: Efficacy models for compound screening. Nat Rev Drug Disc. 2004;3:509–20.
24. LaCrue AN, Scheel M, Kennedy K, et al. Effects of artesunate on parasite recrudescence and dormancy in the rodent malaria model *Plasmodium vinckei*. PLoS One. 2011;6:e26689.
25. Rajendran C, Begam M, Kumar D, et al. Antiplasmodial activity of certain medicinal plants against chloroquine resistant *Plasmodium berghei* infected white albino BALB/c mice. J Parasit Dis. 2014;38:148–52.
26. Ajagbonna OP, Esaigun PE, Alayande NO, Akinloye AO. Anti-malarial activity and haematological effect of stem bark water extract of *Nuclea latifolia.* Bio Res Comm. 2002;14(5):481-486.
27. Visser BJ, Wieten RW, Nagel IM, Grobusch MP. Serum lipids and lipoproteins in malaria - a systematic review and meta-analysis. Mal J. 2013; 12(442):1-16.
28. Georgewill UO, Liverpool E, Adikwu E. Antiplasmodial Assessment of Artemether-lumefantrine-ivermectin on Mice Infected with *Plasmodium berghei*. Asian J of Biol and Life Sci. 2020;9(3) 365-70.
29. Yeo AE, Edstein MD, Shanks GD, Rieckmann KH. Potentiation of the antimalarial activity of atovaquone by doxycycline against *Plasmodium falciparum* in vitro. Parasit Res. 1997; 83:489–91.
30. Prapunwattana P, O'Sullivan WJ, Yuthavong Y. Depression of *Plasmodium falciparum* dihydroorotate dehydrogenase activity in in vitro culture by tetracycline. Mol and Biochem Parasit. 1988;27:119–24.
31. Hayton K, Ranford-Cartwright LC, Walliker D. Sulfadoxine-pyrimethamine resistance in the rodent malaria parasite *Plasmodium chabaud* Antimicrob Agents and Chemother. 2002;46(8):2482-2489.
32. Tärning J. Piperaquine, bioanalysis, drug metabolism and pharmacokinetics. Göteborg, Sweden: Institute of Neuroscience and Physiology, Department of Pharmacology, The Sahlgrenska Academy at Göteborg University; 2007.

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