The Pharmaceutical and Chemical Journal, 2021, 8(1):107-113

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Antiplasmodial activity of artemether-lumefantrine-tinidazole *on Plasmodium berghei* infected mice

Udeme Owunari Georgewill[1], Harold Melford[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, Bayeslsa State, Nigeria
Corresponding author; adikwuelias@gmail.com

Abstract *Introduction:* The impact of malaria scourge has been characterized by daunting challenges including antimalarial drug resistance. This necessitates the search for newer antimalarial drugs using approaches including drug repurposing. This study assessed whether Tinidazole (T) can be repurposed as an antimalaria in combination with artemether/lumefantrine (A/L) in *Plasmodium berghei* infected mice. *Materials and Methods: Plasmodium berghei* infected mice were grouped and orally treated with A/L (2.3/13.7mg/kg), T (28.6 mg/kg), and A/L/T daily in curative, suppressive and prophylactic studies. The negative control (NC) and positive control (MC) were orally treated with 0.9% normal saline (0.2mL) and chloroquine (CQ) (10mg/kg) daily for 4 days, respectively. After drug administration, blood samples were collected and evaluated for parasitemia level, lipid and hematological parameters. *Results*: Significant decreases in parasitemia levels in the curative, suppressive and prophylactic groups were observed in mice treated with T (28.6 mg/kg) ($p<0.05$), A/L (2.3/13.7 mg/kg) ($p<0.01$) and A/L/T ($p<0.001$) when compared to negative control. Mean survival times were significantly increased by T (28.6 mg/kg) ($p<0.05$), A/L (2.3/13.7mg/kg) ($p<0.01$) and A/L/T ($p<0.001$) when compared to negative control. Red blood cells, hemoglobin, packed cell volume, high density lipoprotein cholesterol levels were significantly ($p<0.001$) increased whereas white blood cells, total cholesterol, triglyceride and low density lipoprotein cholesterol levels were significantly decreased by T (28.6 mg/kg) ($p<0.05$), A/L (2.3/13.7mg/kg) ($p<0.01$) and A/L/T ($p<0.001$) when compared to negative control. The antiplasmodial effect of A/L/T differ significantly ($p<0.05$) when compared to positive control. *Conclusion:* Artemether/lumefantrine/tinidazole may be effective against malaria.

Keywords Artemeter; lumefantrine; tinidazole; antiplasmodial; *P. berghei*; mice

Introduction

Malaria is one of the protozoan parasites that have caused significant health challenge to man. It is primarily endemic in sub-tropical and tropical parts of the world. Malaria affects about 3.3 billion people and causes 0.6–1.1 million mortality annually [1]. African countries have the heaviest malaria burden; globally it records 90% of all malaria infections and 92% of malaria associated mortality which significantly impairs economic growth and development [2]. Most deaths associated with malaria are caused by *Plasmodium falciparum* (*P. falciparum*) and occurred in sub-Saharan Africa whereas malaria death in Asia Pacific region and South America is caused by *P. vivax* [3]. Despite measures to curtail malaria infection especially in Africa its impact surges annually. Unfortunately, challenges such as drug resistance, cost and decreased accessibility, affects currently available

antimalarial drugs including artemisinin based combinations, arguably the current hope and primary line of defense against malaria scourge [4,5].

The ongoing search for newer and effective antimalarial drugs that will overcome multi-drug resistant parasites has instigated researchers to invest in various drug discovery and development methods. Drug repurposing is one of the methods of drug discovery that has played a significant role in malaria therapy to date and has attracted renewed interest in recent times for the search for newer antimalarial drugs [6]. The repositioning, or repurposing of already established drugs used for other indications will help discover newer therapies for other diseases. This approach has cutting edge advantages including cost reduction, decreased time and reduced delay in drug discovery and development. It has become an important research method used by the pharmaceutical industry with notable achievement reported in 2004 where 40% of United States Food and Drug Administration (FDA) registered drugs were repurposed [7,8].

Tinidazole (T) (5-nitroimidazole), a widely used drug for the treatment of giardiasis and amoebiasis has a proven safety profile [9, 10]. Despite, its current indications, it has exhibited potential antimalarial activity as captured in emerging studies. Prophylactic testing of T in chick model with *P. gallinaceum* increased survival time from 4 days to 9.5 days [11]. It cured liver stage of infection and delayed relapse in 'Rhesus' macaques (Macaca mulatta) infected with relapsing strain of *P. cynomolgi* and eliminated blood stage of infection in combination with chloroquine (CQ) [10]. In humans with *P vivax*, T monotherapy cleared blood stage infection within 96 hours with no relapse [9]. Due to increased antimalarial activity when combined with CQ, this study assessed whether T can be repurposed for malaria treatment in combination with artemether/lumefantrine (A/L) in *P. berghei* infected mice.

Materials and Methods

Drugs

Chloroquine (CQ) (Evans Medical Nigeria Plc), tinidazole (T) (Norvatis) and artemether/lumefantrine (A/L) (IPAC Laboratory, India) were used for this study. CQ (10mg/kg) [12], A/L (2.3/13.7 mg/kg) [13] and T (28.6 mg/kg) [14] were used.

Experimental animals

Swiss albino mice (25-30g) were used for this study. The mice were purchased from the animal breeding unit of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Rivers State, Nigeria. The mice were kept in plastic cages of 5/group at a temperature of 28.0 + 2.0^{o}C and a 12 hour light/dark cycle. The mice were acclimatized for 2 weeks prior to the experiment with free access to diet and water. The mice were handled according to the directive (2010/63/EU) of the European Union Parliament and the Council on the handling of animals for scientific purposes.

Malaria parasite

A CQ sensitive strain of *Plasmodium berghei (P. berghei)* supplied by Nigerian Institute of Medical Research, Yaba, Lagos was used for this study. The *P. berghei* was maintained by serial blood passage from mouse to mouse every 5-7 days. Blood samples were obtained from donor parasitized mice with a parasitemia of 20-30% into heparinized tubes and diluted with 0.9% normal saline. The mice were infected intraperitoneally (i.p) with the diluted blood sample (0.2 mL) containing 1x10^{7} parasitized erythrocytes. Daily parasitemia levels were monitored using microscopic examination of Giemsa stained thin blood smears. Parasitemia was calculated using the relationship below.

$$\%\ \text{parasitemia} = \frac{\text{Number of parasitized erythrocytes} \times 100}{\text{Total number of erythrocytes}}$$

Evaluation of suppressive antiplasmodial activity

Suppressive antiplasmodial test was evaluated as described by Knight and Peters (1980) [15]. Twenty five mice were inoculated with 1 X 10^{7} *P. berghei* parasitized erythrocytes i.p and randomized into 5 groups (A1-A5) of n=5. After two hours, the mice were treated as follows: Group A1 (Negative control) (NC) was treated with normal saline (0.2mL), group A2 (Positive control) was treated with CQ (10mg/kg) whereas group A3 was treated with T (28.6

mg/kg/day) for 4 days. Group A4 was treated with A/L (2.3/13.7 mg/kg) whereas group A5 was treated with A/L/T for 4 days. On the 5th day, blood samples were collected and thin films produced on microscope slides. The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 min and examined for parasitemia using a microscope. Parasitemia levels were ascertained by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Average percentage inhibitions were calculated using the formula below:

$$\% \text{ Inhibition} = \frac{(\% \text{ parasitemia of untreated control} - \% \text{ parasitemia of treated group}) \text{ x } 100}{\text{parasitemia of untreated control}}$$

Evaluation of curative antiplasmodial activity

Curative antiplasmodial activity was evaluated as described by Ryley and Peter (1970) [16]. Thirty mice were randomized into 6 groups (B1-B6) of n=5. Group BI severed as normal control (MC) whereas B2-B6 served as the experimental groups and were inoculated with 1 X 10^7 *P. berghei* parasitized erythrocytes i.p. Seventy two hours later (Day 3), the groups were treated as follows: Group BI (normal control) and group B2 (Negative control) were treated with normal saline (0.2mL) whereas group B3 (Positive control) was treated with CQ (10mg/kg) daily for 4 days. Group B4 was treated with T (28.6 mg/kg/day), group B5 was treated with A/L (2.3/13.7 mg/kg) whereas group B6 was treated with A/L/T daily for 4 days. Thin films made from collected blood samples were fixed in methanol and stained with 10% Giemsa at pH 7.2 for 10 min. Parasitemia levels were determined microscopically and percentage inhibitions were then calculated as described above.

Evaluation of prophylactic antiplasmodial activity

Prophylactic antiplasmodial activity was determined according to Peters (1967) [17]. Twenty five mice were randomized into 5 groups (CI-C5) of n=5. The mice were pre-treated as follows: Group C1 (Negative control) was treated with normal saline (0.2mL), group C2 (Positive control) was treated with CQ (10mg/kg) whereas group C3 was treated with T (28.6 mg/kg/day) for 4 days. Group C4 was treated with A/L (2.3/13.7 mg/kg) whereas group C5 was treated with A/L/T for 4 days. Thereafter, the mice were inoculated with 1x10^7 *P. berghei* infected erythrocytes i.p and treatment continued. On days 4, 5, 6 and 7 parasitemia levels were determined. Percentage inhibitions were calculated on day 7 as described above.

Determination of mean survival time

The mice were observed daily for mortality. The number of days from the beginning of infection to death for each mouse in the control and experimental groups were recorded. Mean survival time (MST) was calculated using the formula below.

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

Evaluation of lipid and hematological parameters

High-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG). packed cell volume (PCV), red blood cells (RBC), hemoglobin (HB), and white blood cells (WBC) were analyzed using an auto analyzer. LDL-C was calculated using Friedewald equation.

Statistical analysis

GraphPad prism 6.0 statistical software was used for data analyses and data are expressed as mean ± SEM (Standard error of mean). Significant difference among groups were determined using one-way analysis of variance (ANOVA) followed by *Tukey's* post-hoc test. A *p* value less than 0.05; 0.01 and 0.001 was considered significant.

Table 1: Curative antiplasmodial effect of artemether-lumefantrine-tinidazole on *Plasmodium berghei* infected mice

Group	% Parasitemia	% Inhibition	MST
NC	35.9±4.02	-	10.0±1.32
CQ	10.3±0.06[a]	71.0	27.3±2.37[a]
T	14.3±0.73[b]	60.3	20.8±2.44[b]
A/L	10.7±0.37[c]	70.8	25.0±2.20[c]
A/L/T	0.27±0.01[de]	99.1	30.5±2.71[de]

NC: Negative control, CQ: Chloroquine, T: Tinidazole, A/L: Artemether/lumefantrine, A/L/T: Artemether/lumefantrine/tinidazole, MST: Mean survival time, n=5, Data expressed as mean ±SEM, [a] p<0.01 when compared to NC, [b] p<0.05 when compared to NC, [c] p<0.01 when compared to NC, [d] p<0.001 when compared to NC, [e] p<0.05 when compared to CQ, SEM: Standard error of mean

Table 2: Suppressive antiplasmodial effect of artemether-lumefantrine-tinidazole on *Plasmodium berghei* infected mice

Group	**% Parasitemia**	**% Inhibition**	**MST**
NC	24.9±2.02	-	6.02±0.20
CQ	0.59±0.27[a]	97.6%	29.7±1.33[a]
T	8.00±0.26[b]	68.0%	21.5±1.42[b]
A/L	3.38±0.08[c]	86.4%	27.7±2.51[c]
A/L/T	0.03±0.02[de]	99.9%	34.1±3.41[de]

NC: Negative control, CQ: Chloroquine, T: Tinidazole, A/L: Artemether/lumefantrine, A/L/T: Artemether/lumefantrine/tinidazole, MST: Mean survival time, n=5, Data expressed as mean ±SEM, [a] p<0.01 when compared to NC, [b] p<0.05 when compared to NC, [c] p<0.01 when compared to NC, [d] p<0.001 when compared to NC, [e] p<0.05 when compared to CQ, SEM: Standard error of mean

Table 3: Prophylactic antiplasmodial effect of artemether-lumefantrine-tinidazole on *Plasmodium berghei* infected mice

Group	**DAY 4**	**DAY 5**	**DAY 6**	**DAY 7**	**% Inhibition**	**MST**
NC	11.5±1.21	18.2±1.88	23.3±1.58	24.9±2.02		10.7±0.22
CQ	10.4±0.35[a]	10.05±0.33[a]	8.48±0.52[a]	6.82±0.27a	89.6	30.0±3.15[a]
T	10.8±0.92[b]	12.8±0.32[b]	9.05±0.28[b]	7.92±0.40[b]	68.8	23.5±2.77[b]
A/L	9.24±0.26[c]	6.82±0.23[c]	1.46±0.15[c]	2.78±0.08[c]	88.8	27.7±3.20[c]
A/L/T	7.29±0.82[de]	4.82±0.24[de]	1.12±0.05[de]	0.03±0.00[de]	99.9	36.8±3.71[de]

NC: Negative control, CQ: Chloroquine, T: Tinidazole, A/L: Artemether/lumefantrine, A/L/T: Artemether/lumefantrine/tinidazole, MST: Mean survival time, n=5, Data expressed as mean ± SEM, [a] p<0.01 when compared to NC, [b] p<0.05 when compared to NC, [c] p<0.01 when compared to NC, [d] p<0.001 when compared to NC, [e] p<0.05 when compared to CQ, SEM: Standard error of mean

Table 4: Effect of artemether-lumefantrine-tinidazole on lipid profile of *Plasmodium berghei* infected mice

Group	**TG mg/dL**	**CHOL mg/dL**	**HDL-C mg/dL**	**LDL-C mg/dL**
MC	112.0±7.03	160.5±2.46	55.4±1.49	82.7±7.05
NC	300.6±18.9[a]	387.9±17.6 [a]	20.2±1.36 [a]	307.6±18. [a]
CQ	163.0±5.87 [b]	234.1±2.40 [b]	41.3±0.85 [b]	160.2±11.6 [b]
T	240.4±3.03 [c]	307.4±8.24 [c]	30.4±0.58 [c]	228.9±15.0 [c]
A/L	173.7±4.87 [b]	254.3±10.0 [b]	40.1±1.51 [b]	179.5±12.5 [b]
A/L/T	125.6±8.88[de]	187.1±7.22 [de]	52.7±1.11 [de]	109.3±10.1 [de]

MC: Normal control, NC: Negative control, CQ: Chloroquine, T: Tinidazole, A/L: Artemether/lumefantrine, A/L/T: Artemether/lumefantrine/tinidazole, TG: Triglyceride, CHOL: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, n=5, Data expressed as mean± SEM. [a] p<0.001 when compared to MC, [b] p<0.01 when compared to NC, [c] p<0.05 when compared to NC , [d] p<0.001 when compared to NC , [e] p<0.05 when compared to CQ, SEM: Standard error of mean

Table 5: Effect of artemether-lumefantrine-tinidazole on hematological parameters of *Plasmodium berghei* infected mice

Group	RBC x10^6	WBC x10^6	PCV %	HB g/dL
MC	5.74±0.18	6.10±0.15	63.2±6.74	17.6±0.19
NC	3.24±0.13^a	11.2±1.32^a	23.8±3.86^a	8.30±0.61$_a$
CQ	5.42±0.11^b	6.00±0.10^b	50.6±5.74^b	14.9±0.15^b
T	3.70±0.09^c	8.04±0.30^c	37.2±3.66^c	11.8±0.45^c
A/L	5.08±0.13^d	5.96±0.31^d	46.6±4.21^d	14.6±0.22^d
A/L/T	5.68±0.06^{ef}	5.54±0.16^{ef}	60.4±6.81^{ef}	17.0±0.25^{ef}

MC: Normal control NC: Negative control, CQ: Chloroquine, T: Tinidazole, A/L: Artemether/lumefantrine, A/L/T: Artemether/lumefantrine/tinidazole, RBC: Red blood cells, WBC: White blood cells, PCV: Packed cell volume, HB: Hemoglobin, n=5, Data expressed as mean± SEM, ^{a} $p<0.001$ when compared to MC, ^{b} $p<0.01$ when compared to NC, ^{c} $p<0.05$ when compared to NC, ^{d} $p<0.01$ when compared to NC , ^{e} $p<0.001$ when compared to NC , ^{f} $p<0.05$ when compared to CQ, SEM: Standard error of mean

Results

Suppressive and curative tests

In the suppressive and curative tests, treatment with individual doses of T and A/L produced significant reductions in percentage parasitemia levels at $p<0.05$ and $p<0.01$ respectively when compared to negative control. On the hand, most significant ($p<0.001$) reductions in percentage parasitamia levels were observed in mice treated with A/L/T when compared to negative control. The reductions in percentage parasitemia levels in mice treated with A/L/T differ at $p<0.05$ when compared to positive control (CQ) (Tables 1and 2). In the curative test, T, A/L and A/L/T produced percentage parasitemia inhibitions of 60.3%, 70.8% and 99.1% respectively compared to 71.0% produced by CQ. In the suppressive and curative tests, treatment with individual doses of T and A/L significantly increased MST at $p<0.05$ and $p<0.01$ respectively when compared to negative control (Tables 1and 2). Interestingly, most significant ($p<0.001$) increases in MST occurred in mice treated with A/L/T when compared to negative control. The increases in MST time produced by treatment with A/L/T differ at $p<0.05$ when compared to CQ (Tables 1and 2).

Prophylactic test

In the prophylactic antiplasmodial test, treatment with individual doses of T and A/L produced significant time-dependent reductions in percentage parasitemia on days 4, 5, 6, and 7 respectively when compared to negative control. Most significant time-dependent reductions in percentage parasitemia levels on days 4, 5, 6, and 7 were observed in mice treated with A/L/T when compared to negative control (Table 3). The reduction in percentage parasitemia level in mice treated with A/L/T differ at $p<0.05$ when compared to CQ. The percentage parasitemia inhibitions produced by T, A/L and A/L/T on day 7 were 68.8%, 88.8% and 99.9% respectively when compared to 89.6% produced by CQ (Table 3). The MST were observed to be 23.5±2.77, 27.7±3.20 and 36.8±3.71 days for T, A/L and A/L/T respectively compared to 30.0±3.15 days produced by CQ (Table 3).

Effects on hematological and lipid parameters

The negative control shows significant ($p<0.001$) decreases in RBC, HB, PCV and HDL-C levels with significant ($p<0.001$) increases in WBC, TG, CHOL, and LDL-C levels when compared to normal control (Tables 4 and 5). However, treatment with individual doses of T and A/L significantly increased RBC, RBC, HB, PCV and HDL-C levels and significantly decreased WBC, TG, CHOL, and LDL-C levels at $p<0.05$ and $p<0.01$ respectively when compared to negative control (Tables 4 and 5). Interestingly, treatment with A/L/T produced most significant increases in RBC, HB, PCV and HDL-C levels with most significant decreases in WBC, TG, CHOL, and LDL-C levels at $p<0.001$ when compared to negative control. The observed effects produced by A/L/T on RBC, HB, PCV, HDL-C, WBC, TG, CHOL, and LDL-C levels differ from CQ at $p<0.05$ (Tables 4 and 5).

Discussion

Drug repurposing, redirecting, repositioning, or re-profiling is a discovery process used to identify safe compounds or drugs with proven clinical efficacy for the treatment of new or existing diseases. As repurposed drugs are already approved for used clinically, this discovery process causes significant time and cost savings [6]. Drug repurposing includes leveraging on the usefulness of existing drugs by discovering new formulations with varying strengths, combinations and dosing regimens [18]. The search for newer antimalarial drugs through repurposing of existing drugs indicated for other diseases has taken the centre stage in malaria research. This is due to surge in malaria scourge associated with daunting challenges including resistance to current antimalarial drugs [19]. T is a drug primarily indicated for amoebiasis and giardiasis, but has experimentally shown potential antimalarial property. This study assessed whether it can be repurposed as an antimalarial drug in combination with A/L using a mouse model of *P. berghei*-induced malaria. Over the years, curative, suppressive and prophylactic tests have been used and validated as standard experimental procedures for the evaluation of antimalarial drug candidates [20]. In this study, curative, suppressive and prophylactic antiplasmodial assessments of A/L/T showed best decreases in percentage parasitemia levels and increases in percentage parasitemia inhibitions than individual doses of T, A/L and CQ. Malaria is a severe public health problem worldwide. In developing countries, it is a leading cause of mortality with children and pregnant women most affected [2]. One of the primary goals of malaria therapy is absolute reduction in mortality rate or the achievement of zero mortality. MST is experimentally used to assess the abilities of antimalarial drug candidates to reduce mortality in animal models of *plasmodium*-induced malaria [20]. In this study, curative, suppressive and prophylactic antiplasmodial assessments of A/L/T showed the best increases in MST than individual doses of T, A/L and CQ. Malaria induced anemia which is common in malaria endemic regions is a significant public health concern. Malaria induced anemia is characterized by hemolysis of infected and uninfected erythrocytes and bone marrow dyserythropoiesis [21]. In this study, the parasitized untreated mice (Negative control) showed signs of anemia characterized by decreased RBC, HB and PCV levels with increased WBC level. However, decrease in anemia was observed in mice treated with A/L/T characterized by increased RBC, HB and PCV levels with decreased WBC levels best than individual doses of T and A/L. The effects produced by A/L/T on RBC, HB, PCV and WBC levels were also better than CQ. Emerging studies showed the reliance of malaria parasites on cholesterol and phospholipids for survival in their host [22]. Erythrocytic membrane and circulating HDL particles are the sources of cholesterol, whereas erythrocytic membrane is the source of phospholipids for malaria parasites [23]. Some population- based studies reported alterations in serum TG, CHOL, HDL, and LDL in malaria caused by *P. falciparum* especially in endemic regions. [24]. The current study observed increased TG, CHOL and LDL-C levels with decreased HDL-C level in negative control. Interestingly, treatment with individual doses of T and A/L decreased TG, CHOL and LDL-C levels and increased HDL-C levels. However, effects on the aforementioned lipid parameters were best in rats treated with A/L/T.

Conclusion

This study shows that A/L/T may be an effective treatment for malaria. The authors recommend further studies in humans.

Financial Support: None

Conflict of interest: Authors declare no conflict of interest

References

[1]. World Health Organization, 2008. The global burden of disease: 2004 update
[2]. World Health Organisation (WHO) world's malaria report 2007
[3]. World Health Organization 2010, Global report on antimalarial drug efficacy and drug resistance: 2000–2010.
[4]. Dondorp, A.M., Nosten, F., Yi, P., Das, D., Phyo, A.P., Tarning, J. Artemisinin resistance in plasmodium

falciparum malaria. N. Engl. J. Med. 2009; 361 (5), 455–467.

[5]. Ariey, F., Witkowski, B., Amaratunga, C., Beghain, J., Langlois, A.C., Khim, N., A molecular marker of artemisininresistant Plasmodium falciparum malaria. Nature 2014; 505 (7481), 50–55

[6]. Andrews KT , Fisher G, Skinner-Adams TS Drug repurposing and human parasitic protozoan diseases International Journal for Parasitology: Drugs and Drug Resistance 2014; 4; 95–111

[7]. Ashburn TT, Thor KB. Drug repositioning: identifying and developing new uses for existing drugs. Nat. Rev. Drug Discov. 2004; 3, 673–683

[8]. Campas C. Drug repositioning summit: finding new routes to success. Drug News Perspect. 2009; 22, 126–128

[9]. Sarma P: Tinidazole: a new drug in the treatment of vivax malaria. Curr Ther Res 1988, 43:3.

[10]. Deye G, Gettayacamin M, Pranee H, Im-erbsin R, Sattabongkot J, Rothstein Y, Use of Rhesus Plasmodium cynomolgi model to screen for anti-hypnozoite activity of pharmaceutical substances. Am J Trop Med Hyg 2012, 86:931–935.

[11]. 11.Macareo L, Lwin KM, Cheah PY, Yuentraku P, Miller RS and Nosten F, Triangular test design to evaluate tinidazole in the prevention of Plasmodium vivax relapse Malaria Journal 2013, 12:173; 1-6

[12]. Somsak V, Damkaew A, and Onrak P Antimalarial Activity of Kaempferol and Its Combination with Chloroquine in Plasmodium berghei Infection in Mice. Jour of Path 2018; 2018;1- 7

[13]. Sirima SB, Ogutu B, Lusingu JPA, et al. 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 randomised, multicentre, phase 4 trial. Lancet Infect Dis. 2016;16(10):1123-1133.

[14]. Pehrson f, Bengtsson E. Treatment of non-invasive amoebiasis—a comparison between tinidazole and metronidazole. Annals of Tropical Medicine & Parasitology 1984; 78:5,505-508.

[15]. Knight, D.J. and Peters, W. The antimalarial action of N-Benzyl oxydihydrotriazines and the studies on its mode of action. Ann of Trop Med and Par. 1980; **74**: 393-404.

[16]. Ryley, J.F. and Peters, W. The antimalarial activity of some quinolone esters. Ann. Trop. Med. Parasitol. 1970; **84**: 209-222.

[17]. Peters, W. Rational methods in the search for antimalarial drugs. Trans. R. Soc. Trop. Med. Hyg. **1967; 61**: 400-410.

[18]. Nwaka, S., Hudson, A., 2006. Innovative lead discovery strategies for tropical diseases. Nat. Rev. Drug Discov. 5 (11), 941–955.

[19]. Nzila A. The past, present and future of antifolates in the treatment of Plasmodium falciparum infection. J. Antimicrob. Chemother. 2006; 57, 1043–1054.

[20]. Olanlokun JO, Babarinde CO and Olorunsogo O. O. Toxicity of Anchomanes difformis, An Antimalarial Herb in Murine Models Eur Jour of Med Plants 2017; 20(3): 1-13

[21]. White, N.J. Anaemia and malaria. Malar J 17, 371 (2018). 1-17.

[22]. Labaied M, Jayabalasingham B, Bano N, Cha SJ, Sandoval J, Guan G, Coppens I. Plasmodium salvages cholesterol internalized by LDL and synthesized de novo in the liver. Cell Microbiol. 2011;13(4):569–586

[23]. Njoku OU, Ononogbu IC, Nwachukwu DE. Plasma cholesterol, B-carotene and ascorbic acid changes in human malaria. J Commun Dis. 1995;27(3):186–190.

[24]. Fielding PE Fielding CJ. Dynamics of lipoprotein transport in the human circulatory system. New Compr Biochem. 2002;36:527–552.