EFFECTS OF FOUR DIFFERENT CLASSES OF ARTEMISININ-BASED COMBINATION THERAPIES ON LIPID PEROXIDATION AND LIPID PROFILE OF MALARIA-INFECTED MICE.

ISSN: 2141 – 3290
www.wojast.com

[*]EKPO, O. J., ETIM, O. E., NDEM, J. I, AND [1]ANWANA, U. I.

[1]Department of Biochemistry, University of Uyo, Uyo. .
obongjosekacademical21@gmail.com

ABSTRACT

Malaria is caused by the transfer of *Plasmodium* parasite, to a host by an infected female anopheles mosquito. Artemisinin-based combination therapies (ACTs), on the other hand have become the mainstay for malaria treatment. Artemisinin derivatives are highly potent, fast acting antimalarials, but with short half-life, and need to be combined with partner drugs with a longer half-life to clear the remaining parasites after a standard 3-day ACT regimen. Thirty-six, three months old male and female albino mice weighing 25-31g were randomly selected into six groups of six mice per group. Group 1 animals served as normal control (NC). Group 2 animals were infected with *Plasmodium berghei* without being treated with ACTs, and as such served as positive control (PC). The test groups 3, 4, 5 and 6 animals were infected with *Plasmodium berghei* and treated with artesunate-amodiaquine (AA), artemether-lumefantrine (AL), artesunate-mefloquine (AM), and dihydroartemisinin-piperaquine phosphate (DPP) respectively, at therapeutic doses. At the end of the three days treatment, the animals were sacrificed using chloroform anaesthesia and the blood samples were collected by cardiac puncture for analyses using standard methods. Artesunate-amodiaquine and artemether-lumefantrine caused significant ($p<0.05$) increase in serum malondialdehyde concentration when compared to the positive control. The groups treated with artesunate-amodiaquine, artemether-lumefantrine, artesunate-mefloquine and dihydroartemisinin-piperaquine phosphate showed significant ($p<0.05$) decrease in the concentrations of total cholesterol and low-density lipoprotein when compared with the positive control. The group treated with artesunate-mefloquine indicated a significant ($p<0.05$) reduction in the concentrations of triglycerides and very low-density lipoprotein when compared to the positive control. Artesunate-amodiaquine and artemether-lumefantrine treated groups indicated increased significance ($p<0.05$) in the concentration of high-density lipoprotein when compared to the positive control. Hence, this study revealed that the treatment of malaria-infected albino mice with ACTs generally elevate the peroxidation of lipid and do not significantly contribute to the amelioration of lipid profile.

INTRODUCTION

Malaria is the most lethal parasitic disease in the world, annually affecting approximately five hundred million people mostly in African sub-Saharan countries (WHO, 2000; Snow *et al*., 2005). *Plasmodium berghei* and *Plasmodium vinckei* are considered a comparable genetic model to humans. There is a high degree of genomic conservation, up to 99% (Pennacchio, 2003), and it is well established that mice also exhibit natural difference in susceptibility to malarial infection (Greenberg *et al*., 1954). Patients with malaria present hypocholesterolemia, decreased levels of high-density lipoproteins (HDL) and low-density lipoproteins (LDL), which are accompanied by increased levels of triglycerides, and very low-density lipoproteins (VLDL). Such lipid abnormalities are transient, occurring in the most prevalent species of *Plasmodium* as well as in complicated or noncomplicated cases (Visser *et al.,* 2013).

Animal (on rats) studies have suggested toxicity on the haematopoietic system with reticulocytes reversible decrease, but clinical observations point out to a lower toxicity in

malaria patients compared to healthy volunteers (Clark, 2012). However, research on the effects of the four different classes of ACTs on the lipid peroxidation and lipid profile of malaria-infected mice is yet to be carried out.

The *Artemisia annua* plant (*Artemisia*) is known to be the most ancient antimalarial treatment, having been used in China for over 2000 years. It contains artemisinin, a substance which eliminates the blood-stage parasites more rapidly than any other drug and works well against *Plasmodium falciparum* species that are resistant to other drugs. This drug produces free radicals in contact with iron, a common metal in the body, especially within erythrocytes (Grahame-Smith, 2004). Artemisinin is a saturated endoperoxide lactone molecule and has been used by the Chinese for two millenniums as a folk remedy against fever (Mpiana *et al*., 2007). The artemisinins produce fast recrudescence when used alone due to their short half-lives. ACTs combine artemisinin derivatives with other antimalarials, including quinoline compounds, such as amodiaquine and mefloquine. The quinolines act mainly by inhibiting hematin polymerization, thus intoxicating the parasite with the ferriprotoporphyrin groups generated by hemoglobin degradation (Vennerstrom *et al*., 1999). Other antimalarials used in ACTs, for example, pyrimethamine and proguanil, inhibit the tetrahydrofolic acid cycle and thus eliminate an important cofactor for DNA synthesis. Chuljerm *et al.* (2021), observed high increases in lipid peroxidation products, including thiobarbituric acid-reactive substances (TBARS) (e.g MDA) in the plasma and livers of *Plasmodium berghei* ANKA mice ($p<0.05$), treated with deferiprone, deferiprone-resveratrol, and pyrimethamine, when compared with normal mice.

MATERIALS AND METHOD

Chemicals and Equipment

Chemicals and reagents of analytical grade were used for this study. The assay kits for the estimation of serum malondialdehyde (MDA), total cholesterol (TC), high density lipoprotein (HDL), and triglyceride (TG) were obtained from Fortress Diagnostics, unit 2C Antrim Technology Park, Antrim, United Kingdom. The equipment and glasswares used in this research are spectrophotometer, centrifuge, light microscope, refrigerator, automated micropipette, test tubes, syringes, hand gloves, plain tubes, slides, slide box and plastic cages.

Experimental Animals

Thirty six albino mice were used in this research, 10-15 weeks old. Swiss albino males and females of weight ranging from 25-31g, obtained from the Animal House, Faculty of Basic Medical Sciences, University of Uyo, Uyo. The experimental animals were fed *ad libitum* on a commercial diet, housed in plastic cages with wire guaze for sufficient ventilation and with provision for feed and water. Room temperature of 26 ± 2°C and the relative humidity of 46% were maintained. The albino mice were allowed to acclimatize for 7 days and were handled in accordance with the guidelines for laboratory animal use of the University of Uyo, Uyo.

The parasite used in this study is chloroquine-sensitive *Plasmodium berghei,* NK 65 strain, obtained from the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, and it constituted the blood of albino mice already infected with *plasmodium berghei*, serving as the donor mice for further use in this experiment. The characteristics of *plasmodium berghei* are quite identical to that of *plasmodium falciparum* known to be the common cause of malaria in humans. The parasites were maintained by serial passage of blood from infected mice to non-infected ones to induce malaria in them.

Drug Samples and Drug Preparation

P-Alaxin (containing dihydroartemisinin-piperaquine phosphate), Artequin (containing artesunate-mefloquine), Lumartem (containing artemether-lumefantrine) and Camosunate (containing artesunate-amodiaquine), were purchased from the Contour Pharmacy, Abak. Standard recommended therapeutic doses (according to the weight of the experimental animals) of each ACTs suspended in vehicles were administered orally to the different groups of the

experimental animals for three days. The ACTs were administered orally *via* an oral carnula and new stock solution, of the drugs were prepared, each day of the administration.

Experimental Design and Treatment of Animals

A total of thirty six albino mice were divided into six different groups of six per group. Group 1, acting as the normal control, was uninfected with the parasite and was given normal rat pellets and water. Group 2, acting as the positive control, was infected with the parasite but untreated. Group 3 was infected with the parasite and then treated with 1.42 mg/kg body weight of Artesunate and 4.29mg/kg body weight of Amodiaquine twice a day for three days. Group 4 was infected with the parasite and then treated with 1.14mg/kg of Artesunate body weight and 6.86mg/kg body weight of Lumefantrine twice a day for three days. Group 5 was infected with the parasite and then treated with 2.86mg/kg body weight of Artesunate and 3.57mg body weight of mefloquine once a day for three days. Group 5 was infected with the parasite and then treated with 1.71mg/kg body weight of dihydroartemisinin and13.71mg/kg body weight of Piperaquine Phosphate once a day for two days followed by the administration of two-third of the dosage, on the third day.

Animal Sacrifice, Blood Samples Collection and Sera Preparation for Analyses

At the end of the third day administration, the mice were fasted overnight (12hrs) and induced with anesthesia by dropping each mice in a transparent desiccators, saturated with chloroform vapour. Incision was performed on the abdomen of the weakened mice. Blood samples were collected via cardiac puncture, using sterile syringes and needles into sterile plain tubes for sera preparation.

Biochemical Analyses

Estimation of total cholesterol (TC) was carried out according to the method of Richmond (1973). Estimation of triacylglycerol (TG) was carried out according to the method of Trinder (1969). Estimation of HDL cholesterol was carried out according to the method of Richmond (1973). Lipid peroxidation was assessed by measuring MDA, an end product of fatty acid peroxidation, according to the method of Ohkawa *et al.* (1979).

Statistical Analysis

Differences between groups were determined by one-way analysis of variance (ANOVA), and post hoc testing was performed for intergroup comparisons using the least significant difference (LSD). Data presented are mean ± standard error of mean (SEM) and were analyzed using Statistiscal Package for Social Sciences (SPSS) software for windows. $P< 0.05$ was considered significant.

RESULTS AND DISCUSSION

The malaria therapy currently includes artemisinin-based combination therapy as first- and second-line treatment in most endemic countries which consists of artemisinin combination (or one of its derivatives) with other drug (s) (or two other antimalarials) referred to as partner drugs, and antimalarial drug resistance is one of the greatest threat to the control and elimination of malaria globally (WHO, 2018a). The drug combinations in the ACT not only have different mechanism leading to their improved efficacy but also, the chances of emergence of drug resistance to each component drugs are lower (Ashley *et al.*, 2018). Hence, the results (Table 1) have shown the efficacy of ACTs against the parasites.

Table 1: Four classes of ACTs and their Percentage Parasitemia Clearance

Group Parasitemia Clearance	Pre-Treatment	Post-Treatment	%
NC	0.00±0.00	0.00 ±0.00	0.00
PC	17868.00±3030.41	56261.29±5565.44	-----
AA	25368.00 ±4987.35	71.46±41.95	99.72
AL	12924.00 ±3382.21	188.41±107.08	98.54
AM	29022.00±6283.26	885.51±467.59	96.95
DPP	17688.00±2486.93	294.13±108.70	98.34

NC=normal control, PC=positive control, AA=artesunate amodiaquine, AL=artemether lumefantrine, AM=artesunate mefloquine, DP=dihydroartemisinin piperaquine phosphate

Table 2: Lipid peroxidation (MDA Concentration)

GROUPS	MDA(uMol)
NC	1.35± 0.20
PC	1.56 ± 0.22
AA	$4.88\pm0.56^{a,b}$
AL	$4.58\pm0.53^{a,b}$
AM	$2.16\pm0.38^{c,d}$
DPP	$1.15\pm0.18^{c,d}$

NC=normal control, PC=positive control, AA=artesunate amodiaquine, AL=artemether lumefantrine, AM=artesunate mefloquine, DP=dihydroartemisinin piperaquine phosphate

Data are presented as Mean ± Standard Error of Mean. a = significantly different from Group 1 ($p < 0.05$); b = significantly different from Group 2 ($p < 0.05$); c = significantly different from Group 3 ($p < 0.05$); d= significantly different from Group 4 ($p < 0.05$), e = significantly different from Group 5 ($p < 0.05$).

Table 3: Lipid Profile Concentrations

GROUPS	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL(mg/d)
NC	90.73± 2.72	51.45± 1.32	16.17±0.89	64.28±3.08	10.29±0.26
PC	96.79 ± 0.58^{a}	54.54 ±1.27	13.4 ± 0.66^{a}	72.42 ± 0.35^{a}	10.91±0.25
AA	90.21 ± 1.55^{b}	55.54 ± 0.96^{a}	16.77 ± 0.68^{b}	62.34 ± 1.66^{b}	11.11 ± 0.19^{a}
AL	$83.58\pm3.07^{a,b,c}$	51.07 ± 1.20^{c}	$20.02\pm1.32^{a,b,c}$	$53.35\pm2.04^{a,b,c}$	10.22 ± 0.24^{c}
AM	$76.37\pm1.80^{a,b,d}$	$44.51\pm1.53^{a,b,d}$	15.48 ± 0.52^{d}	$51.98\pm1.70^{a,b,c}$	$8.90\pm0.31^{a,b,c,d}$
DPP	$78.81\pm 2.06^{a,b,c}$	$55.87\pm1.44^{a,d,e}$	14.61 ± 0.93^{d}	$53.03\pm1.93^{a,b,c}$	$11.18\pm0.29^{a,d,e}$

NC=normal control, PC=positive control, AA=artesunate amodiaquine, AL=artemether lumefantrine, AM=artesunate mefloquine, DP=dihydroartemisinin piperaquine phosphate

Data are presented as Mean ± Standard Error of Mean. a = significantly different from Group 1 ($p < 0.05$); b = significantly different from Group 2 ($p < 0.05$); c = significantly different from Group 3 ($p < 0.05$); d= significantly different from Group 4 ($p < 0.05$), e = significantly different from Group 5 ($p < 0.05$).

Artemisinin-based combination therapy (ACT) has been adopted as a strategy to mitigate multidrug resistance to antimalarial monotherapies. ACT combines the rapid and effective but rather short plasma half-life antimalarial action of an artemisinin derivative with a longer acting partner drug (Kavishe *et al*., 2017).

The level of parasitemia (parasite count) was observed under the microscope using Giemsa-stained thin blood films (WHO, 2000), and presented in Table 1. The result showed a high percentage clearance in the four treatment groups after being administered with the ACTs. Additionally, this finding indicated that the ACTs generally have a high percentage clearance on parasitemia when compared with the positive control group. However, the group treated

with Artesunate-Amodiaquine indicated the highest percentage clearance. This highest percentage clearance, is consistent with the view that parasitemia increases progressively after inoculation or infection until the point of death in the absence of suitable treatment (Breman *et al*., 2001; Trampuz *et al.*, 2003).

Malondialdehyde (MDA) is a reactive aldehyde that is synthesized as one of the end products of the process of lipid peroxidation. As presented in Table 2, a significant decrease in the concentration of Malondialdehyde in the positive control was observed when compared with the groups treated with Artesusunate-Amodiaquine and Artemether-Lumefantrine. This suggests that Artesunate-Amodiaquine and Artemether-Lumefantrine increase lipid peroxidation process when used in the treatment of malaria due to increased concentration of MDA in the serum. This result is in agreement with Pandey *et al.* (2001), Bolchoz *et al.* (2002) and Haynes *et al*. (2004), who reported that even antimalarial drug therapy constitutes a source of oxidation, as many drugs such as chloroquine, primaquine and derivatives of artemisinin are inducers of free radical production. The oxidation process of fatty acids, termed lipid peroxidation, appears to originate from the cardiolipin oxidation in the apoptosis context. This is because a prime event in malaria infection is increased production of highly reactive oxygen species (ROS) as part of the host defense (Wozencraft *et al*., 1984). Dey *et al*. (2009), attributed the hydroxyl radical with the function of triggering the process in hepatocytes. It was also observed that there was no significant difference in the concentration of MDA in the positive control when compared with the normal control and the groups treated with Artesunate-Mefloquine and Dihydroartemisinin-Piperaquine Phosphate. This finding is not consistent with the report that women treated with antimalarial drugs, lipid peroxidation levels were even higher, with a more intense GSH and ascorbate decrease than in women not treated with these drugs (Akanbi *et al.,* 2010). However, higher levels of lipid peroxidation (malondialdehyde, total plasma peroxides, and oxidative stress index), lower antioxidant (reduced glutathione, and total antioxidant capacity), and nitric oxide were observed in malaria patients with or without antimalaria therapy when compared to their respective controls (Nsonwu-Anyanwu *et al*., 2019).

Lipids and lipoproteins are risk factors for coronary heart disease. It has been demonstrated that high levels of serum total cholesterol (TC), triglycerides (TG), LDL cholesterol, very-low-density lipoprotein (VLDL) and low concentration of HDL cholesterol are significantly associated with coronary heart disease (George and Ludvik, 2000). High Density Lipoprotein (HDL) helps to extract excess cholesterol deposited in blood vessel walls and deliver it back to the liver for elimination through the gastrointestinal tract. HDL cholesterol therefore aids to keep blood vessels dilated, thereby promoting better blood flow. It also reduces blood vessel injury through its antioxidant and anti-inflammatory functions, among other effects. HDL cholesterol carries “old” cholesterol that has been discarded by cells back to the liver for recycling or excretion. The main function of low density lipoprotein cholesterol is to transport cholesterol from the liver to the tissues that incorporate it into the cell membranes. The oxidation of LDL cholesterol is believed to have a central role in atherogenesis. Oxidized LDL cholesterol may be involved in atherogenesis by inducing smooth muscle cell proliferation. Serum cholesterol plays an important role in atheromatous disease. In conditions with elevated concentrations of oxidized LDL particles, especially small LDL particles, cholesterol promotes atheroma plaque deposits in the walls of arteries, a condition known as atherosclerosis, which is a major contributor of the disease. In contrast, HDL particles have been the only identified mechanism by which cholesterol can be removed from atheroma. Therefore, an increased concentration of HDL-C as is observed in our study, correlates with lower rates of atheroma progression, even regression. In our study, we observed higher HDL-cholesterol than LDL-cholesterol. There is a universal trend that lower total cholesterol levels tend to correlate with lower rate of atherosclerotic event. However, the primary association of atherosclerosis with cholesterol has always been specific with cholesterol transport patterns, but not on total cholesterol level. For instance, if total cholesterol can be low, yet made up of small LDL and

small HDL particles and atheroma growth rates are high. In contrast, however, if LDL particle number is low and a large percentage of the HDL particles are large, then atheroma growth rates are usually low, even negative, for any given total cholesterol concentration (Adekunle *et al.,* 2007).

The effects of the four classes of ACTs on the lipid profile are presented in Table 3. There was significant decrease in the concentration of TC in the groups treated with Artesunate-Amodiaquine, Artemether-Lumefantrine, Artesunate-Mefloquine and Dihydroartemisinin-Piperaquine Phosphate when compared with the positive control.This is in contrast with the report of Kullu *et al*. (2018), who reported a significant decrease in the level of total cholesterol in *plasmodium falciparum* malaria infected patient when compared with the control. There was significant decrease in the concentration of TG in group treated with Artesunate-Mefloquine when compared with the positive control. This finding agrees with Visser *et al.* (2013), who reported that triglycerides are significantly higher in malaria patients when compared with healthy controls, but these differences become non-significant when compared to symptomatic controls. In patients with severe malaria, triglyceride levels were found to be higher compared to triglyceride levels in patients with uncomplicated malaria Mfonkeu *et al.* (2010). There was no significant difference in the level of TG in Artesunate-Amodiaquine, Artemether-Lumefantrine and Dihydroartemisinin-Piperaquine Phosphate when compared with the positive control. This finding is not consistent with Kullu *et al.* (2018), who reported that serum triglyceride was significantly higher in *Plasmodium falciparum* malaria than in control group.

There was significant decrease in the concentration of High Density Lipoprotein (HDL) in the positive control when compared with the normal control. This finding is in line with Faucher *et al.* (2002), who reported that malaria infection produces moderate changes in plasma lipid profile in man, with typical decline in HDL concentration. However, there was a significant increase in the concentration of HDL in the artesunate amodiaquine and and artemether lumefantrine treated groups when compared with the positive control. This increase in the concentration of HDL is consistent with the report that serum HDL is significantly decreased in *Plasmodium falciparum* malaria than in control group (Kullu *et al*., 2018).

Significant reduction in the concencentration of Low Density Lipoprotein (LDL) was indicated in the Artesunate-Amodiaquine, Artemether-Lumefantrine, Artesunate-Mefloquine and Dihydroaremisinin-Piperaquine Phosphate treated groups when compared with the positive control. This is not consistent with Das *et al.* (1996), and Mfonkeu *et al.* (2010) who reported a significant larger decline in LDL in patients with severe malaria compared with patients with uncomplicated malaria. There was no significant difference in the concentration of very low density lipoprotein (VLDL) in the the groups treated with Artesunate-Amodiaquine, Artemether-Lumefantrine and Dihydroartemisinin-Piperaquine Phosphate when compared with the positive control. This is in contrast with Kullu *et al.* (2018), who reported that VLDL was higher in *Plasmodium falciparum* malaria than in the control group.

CONCLUSION

This study shows that the treatment of malaria-infected albino mice with ACTs generally result in the elevation of the peroxidation of lipid and do not significantly contribute to the amelioration of lipid profile.

REFERENCES

Adekunle, A. S., Adekunle, O. C. and Egbewale, B. E. (2007). Serum status of selected biochemical parameters in malaria: *An animal model. Biomedical Research,* 18 (2): 109-113.

Akanbi, O. M., Odaibo, A. B. and Ademowo, O. G. (2010). Effect of antimalarial drugs and malaria infection on oxidative stress in pregnant women. *African Journal of Reproductive Health,* 14:10.

Ashley, E. A., Phyo, A. P., Woodrow, C. J. (2018). Malaria. *Lancet*, 391:1608–1621.

Bolchoz, L. J., Gelasco, A. K., Jollow, D. J. and McMillan, D. C. (2002). Primaquine-induced hemolytic anemia: Formation of free radicals in rat erythrocytes exposed to 6-methoxy-8-hydroxylaminoquinoline. *Journal of Pharmacology and Experimental Therapeutics,* 303:1121–1129.

Breman, J. G., Egan, A. and Keusch, G. (2001).The intolerable burden of malaria: A new look at thenumbers. *American Journal of Tropical Medicine and Hygiene*, 64:4–7.

Chuljerm, H., Maneekesorn, S., Somsak, V., Ma, Y., Srichairatanakool, S., and Koonyosying, P. (2021). Antimalarial, and Anti-
lipid peroxidation Activities of Deferiprone-Resveratrol Hybrid in *Plasmodium berghei*-infected mice. *Biology*, 10(911): 2-14.

Clark, R. L. (2012). Effects of artemisinins on reticulocyte count and relationship to possible embryotoxicity in confirmed and unconfirmed malarial patients. *Birth Defects Research A Clinical and Molecular Teratology*, 94:61–75.

Das, B. S., Thurnham. D. I. and Das, D. B. (1996). Plasma alpha-tocopherol retinol and carotenoids in children with *falciparum* malaria.*American Journal of Clinical Nutrition,* 64: 94–100.

Dey, S., Guha, M., Alam, A., Goyal, M., Bindu, S., Pal, C., Maity, P., Mitra, K. and Bandyopadhyay, U. (2009). Malarial infection develops mitochondrial pathology and mitochondrial oxidative stress to promote hepatocyte apoptosis. *Free Radical Biology,* 46:271–281.

George, P. and Ludvik, B. (2000). Lipids and diabetes. *Journal of Clinical Basic Cardiology,* 3:159-162

Grahame-Smith, D. G. and Aronson, J. K. (2004).Tratado de Farmacologia Clínica Farmacoterapia.3rd ed. Guanabara Koogan; Rio de Janeiro, RJ, Brazil.

Greenberg, J., Nadel, E. M. and Coatney, G. R. (1954).Differences in survival of several inbred strains of mice and their hybrids infected with *Plasmodium berghei*. *Journal of Infectious Dis*ease, 95: 114–116.

Haynes, R. K. and Krishna, S. (2004). Artemisinins: Activities and actions. *Microbe Infections*, 6:1339–1346.

Kavishe, R. A, Koenderink, J. B. and Alifrangis, M. (2017). Oxidative stress in malaria and artemisinin-based combination therapy: Pros and Cons. *The FEBS Journal*, 284:16.

Kullu, B. K., Majhi, C., Pradhan, B. and Swain, D. (2018). Lipid profiles among *P. falciparum* infected, non-malarial febrile patients and volunteers. *International Journal of Advances in Medicine,* 5(3):556–560

Mfonkeu, J. B., Gouado, I., Kuate, H. F., Zambou, O., Combes, V. and Grau, G. E. (2010). Biochemical markers of nutritional status and childhood malaria severit in Cameroon. *British Journal of Nutrition,* 104:886–892.

Mpiana, P. T., Mavakala, B. K. and Zhi-Wu, Y. (2007).Interaction of artemisinin based antimalarial drugs with hemin in water-DMSO mixture. *International Journal of Pharmacology*, 3: 302–310.

Nsonwu-Anyanwu, A.C, Osuoha, U. O, Nsonwu, M.C, and Usoro, C.A.O. (2019). Antimalaria therapy and changes in oxidative stress indices in falciparum malaria infection in Calabar Metropolis, Nigeria. *Tropical Journal of Pharmaceutical Research,* 18(11):2431-2437.

Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem,* 95:351–358.

Pandey, A. V., Bisht, H., Babbarwal, V. K., Srivastava, J., Pandey, K. C. and Chauhan, V. S. (2001). Mechanism of malarial haem detoxification inhibition by chloroquine. *Biochemistry Journal,* 355:333–338.

Pennacchio, L. A. (2003). Insights from human/mouse genome comparisons.*Mammal Genome*, 14: 429–436.

Richmond, W. (1973). Cholesterol Enzymatic Colorimetric Test: CHOP PAP-Method of Estimation of Total Cholesterol in Serum. Clinical Chemistry, 9:1350-1356.

Snow, R. W., Guerra, C. A., Noor, A. M., Myint, H. Y. and Hay, S. I. (2005). The global distribution of clinical episodes of *Plasmodium falciparum* malaria.*Nature*, 434: 214-217.

Trampuz, A., Jereb, M., Muzlovic, I. and Prabhu, R. M. (2003). Clinical review: Severe malaria. *Critical Care*, 7: 315–323.

Trinder, P. (1969). Triglycerides Estimation in Serum. *Annals of Clinical Biochemistry*, 6:26

Vennerstrom, J. L. , Vippagunta, S. R., Dorn, A., Matile, H., Bhattacharjee, A.K, Karle,. M, Ellis, W. Y. and Ridley, R. G. (1999). Structural specificity of chloroquine-hematin binding related to inhibition of hematin polymerization and parasite growth. *Journal of Medicinal Chemistry,* 22:4630-4639.

WHO, (2000). Severe *falciparum* malaria.*Transactions of Royal Society Tropical Medicine and Hygiene,* 94:1–90.

WHO, 2018a. World Malaria Report 2018, p. 210. Geneva, Switzerland.

Wozencraft, A. O., Dockrell, H. M., Taverne, J. G., Targett, A. and Playfair, J. H. (1984). Killing of human malaria parasites by macrophage secretory products. *Infectious Immunology*, 43: 664–689