Biolife 1(2):-70-77, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE ORIGINAL ARTICLE

ANTIOXIDANT BASED COMBINATION THERAPY IN MALARIA: *IN VIVO* STUDY IN *PLASMODIUM BERGHEI* INFECTED MICE

[1]Iribhogbe O.I, [2]Agbaje E.O, [2]Oreagba I.A, [3]Aina O.O, [4] Ota A. D.

[1]Department of Pharmacology & Therapeutics College of Medicine,
Ambrose Alli University Ekpoma. Edo State Nigeria.
[2]Department of Pharmacology College of Medicine of the University of Lagos.
[3] Malaria Research Unit, Nigerian Institute of Medical Research, Yaba Lagos State.
[4] Department of Physiology College of Medicine of the University of Lagos.

E-mail: osedeignis@gmail.com, oignis@yahoo.com

ABSTRACT

The emergence of resistance to previously established antimalarial agents, have created the need for a continued effort geared towards the discovery of newer agents. The present study involves an *in vivo* evaluation of the potential effect of selected antioxidant micronutrient combination in the therapeutics of malaria. In this study, rodent malaria model; *Plasmodium berghei* NK-65 strain (chloroquine sensitive) and chloroquine resistant ANKA strain was used. In the first stage of the experiment, a 4 day curative synergistic test was conducted using 45 mice of either sex weighing 20.05±0.02 g which were inoculated intraperitoneally with 1 x 10^7 million *Plasmodium berghei* infected erythrocyte and were administered with varying combination of selected micronutrients for 4 days, 72 hours post inoculation. The second stage involved the use of 45 mice of either sex; curative synergistic study using chloroquine resistant ANKA strain was conducted. Similarly, varying combination of micronutrients with standard antimalarial agents was administered for 4 days. Synergistic schizonticidal activity was most marked in the vitamin A + E combination group (94.52%) when compared with any other micronutrient combination group after 4 days treatment of established infection. This was closely followed by the vitamin A + selenium combination group (94.43%). The mean difference in parasitemic levels was significant between groups ($F = 2.59$; $P < 0.05$). Conclusively, antioxidant micronutrient combination has potential benefit of being used as adjuvant in malaria therapeutics.

Key words : Adjuvant, Antioxidant Micronutrients, Combination Therapy, Drug Resistance.

INTRODUCTION

Several efforts have been in line to discover and develop new antimalarial agents. This has increased in recent years as a result of the recognition of its global significance. In addition, high level of commitment from public-private partnership aimed at discovery, development and delivery of new drugs has also increased. Despite these strategies, morbidity and mortality from malaria is still on the rise in Africa and other developing parts of the world. This is primarily due to increasing ineffectiveness of common first line agents like chloroquine and sulphadoxine-pyrimethamine combination in addition to the inaffordability of present alternative medications such as the artemisinin based combination therapy (ACT). However, non-artemisinin based combinations such as amodiaquine and sulfadoxine-

pyrimethamine showed excellent efficacy in East Africa despite existing high level of resistance to individual agents (Dorsey et al., 2002; Schellenberg et al., 2002; Staedke et al., 2001). There is the possibility that chloroquine can be used in combination therapy particularly in areas where its use has been discontinued over a long time (Kublin et al., 2003). According to Kublin et al., (2003) the re-emergence of chloroquine sensitivity in Malawi was due to prolonged discontinuation of its use for about a decade. Artemisinin analogs such as artesunate and artemether has shown great efficacy as antimalarial agents. However, despite the rapidly potent antimalarial activity of these agents their use as mono-therapeutic agents is limited by their short half lives and the occurrence of late recrudescence. Hence, they are presently used as combination therapy with other agents that have longer half lives (WHO, 2003). New natural products are currently being screened for antimalarial activity this include evaluation of micronutrient and products from plant extracts (Tagboto and Townson, 2001). The present study was designed to evaluate the potential therapeutic efficacy of antioxidant micronutrient based combination in malaria treatment using mouse model.

MATERIALS & METHODS

Materials

Chemicals and equipments: Heparinised capillary tubes, Light Microscope (Olympus, Japan), EDTA bottles, Feeding trochars, Syringes (1ml, 5mls), Cotton wool, Microscopic slides (Olympus, China), Hand gloves, Giemsa stain (Sigma), 98% Methanol (Sigma) and Tween 80 (sigma).

Drugs: Vitamin A (Clarion Medical Pharmaceuticals, Nigeria), Vitamin E (Clarion Medical Pharmaceuticals, Nigeria), Zinc gluconate (Mason Vitamins Incorporated USA), Selenium-organic (Mason Vitamins Incorporated USA), Chloroquine (Emzor Pharmaceuticals, Nigeria), Pyrimethamine (Glaxo Smith Klime, Nigeria) and Artesunate (Emzor Pharmaceuticals, Nigeria). The present study was conducted between January and August, 2010.

Preparation of Animals

Ninety in bred pure Swiss albino mice of either sex weighing between 18- 25g were used for the study. They were obtained from the animal house of the Nigerian Institute of Medical Research, Yaba Lagos State and housed in stainless steel cages with wire screen top. The animals were about 7-8 weeks old and were maintained on commercial feeds (Vital feeds, Jos) and tap water *ad libitum* for the entire duration of the study. The mice were allowed to acclimatize for 1 week in the laboratory environment under a controlled temperature of 20^0 C and at optimum humidity before being subjected to the experiment (Obernier and Baldwin, 2007). Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from the cages daily.

Preparation of Inoculum of Chloroquine Sensitive Strain of *Plasmodium berghei*

Plasmodium berghei NK 65 strain maintained in the laboratory of Nigerian Institute of Medical Research, Yaba by serial blood passage from mouse to mouse was used for the study. Donor mouse with a rising parasitaemia of 20 -30% confirmed by thin and thick blood film microscopy was used. Blood (0.2ml) was collected in a heparinized tube from the auxiliary plexus of veins in the donor mouse using heparinized capillary tubes. The blood was diluted with 5ml of Phosphate buffer solution (PBS) pH 7.2 so that each 0.2 ml contained approximately 1×10^7 infected red cells (Peter et al., 1975;David et al., 2004). Each animal received inocula of about 10 million parasites per kilogram body weight, which is expected to produce a steadily rising infection in mice.

Study Design/Drugs and Micronutrient Administration

The study was divided into two stages:

Stage 1:

In this stage, the aim was to determine the curative effect of different antioxidant micronutrient combinations using the 4 day

curative test as described by Agbaje and Onabanjo, (1994); David et al., 2004); Adzu et al., (2007). The animals in group A were administered a single oral dose of 25mg/kg chloroquine as a reference drug (Tekalign et al., 2010). The antioxidant micronutrients were administered orally as follows; vitamin A (60mg/kg), vitamin E (100mg/kg), zinc (100mg/kg), selenium (1mg/kg) using doses based on LD_{50} values as reported by Schrauzer, (2000); Oncu et al., (2002); Oreagba and Ashorobi, (2006). Group B animals were administered 0.2 ml of distilled water orally, group C animals with 0.2ml of the vehicle tween 80, group D animals were treated with a single oral dose of vitamin A (60mg/kg) and a single oral dose of vitamin E (100 mg/kg), group E animals with a single oral dose of vitamin A (60 mg/kg) and selenium (1mg/kg), group F animals were treated with a single oral dose of vitamin A (60 mg/kg) and zinc (100 mg/kg), group G animals received vitamin E (100 mg/kg) and zinc (100 mg/kg), group H were administered oral doses of vitamin E (100 mg/kg) and selenium (1mg/kg) and group I received oral doses of zinc (100 mg/kg) and selenium 1mg/kg respectively for 4 days (Table 1a). On day four post infection, daily smears for thin blood film were made to ascertain % chemosupression, parasite clearance time (PCT), recrudescence time (RT) and mean survival time. % Chemosupression was calculated by using the formulae by (Peters et al, 1977; Peter and Anatoli, 1998; David et al., 2004).

Stage 2

In this stage, the synergistic or additive antimalarial effects of different combinations of selected antioxidant micronutrients and some standard antimalarial drugs were assessed. The inoculum containing the chloroquine resistant strain of *Plasmodium berghei* (ANKA strain) was used. A four day curative test was done according to previously stated schedule. Group A animals were dosed orally with a single dose of chloroquine 25mg/kg, group B animals were dosed orally with 4 mg/kg artesunate, group C animals were dosed orally with distilled water

Table 1a: Drug Administration (per os) in the Animals:

Groups	Drugs/Micronurients	Dosage
A= parasitized mice (positive control)	Chloroquine sulphate	25 mg/kg
B= parasitized mice (negative control group)	Distilled water	0.2 mls
C= parasitized mice (vehicle control group)	Tween 80	0.2 mls
D= parasitized mice (test group 1)	Vitamin A+E	60mg/kg/100mg/kg
E= parasitized mice (test group 2)	Vit A+Selenium	60mg/kg/1mg/kg
F= parasitized mice (test group 3)	Vit A+Zinc	60mg/kg/100mg/kg
G= parasitized mice (test group 4)	Vit E+Zinc	100mg/kg/100mg/kg
H= parasitized mice (test group 5)	Vit E +Selenium	100mg/kg/1mg/kg
I= parasitized mice (test group 6)	Zinc +Selenium	100mg/kg/1mg/kg

Table 1b: Drug Administration (per os) in the Animals:

Groups	Drugs/Micronurients	Dosage
A= parasitized mice (positive control)	Chloroquine sulphate	25 mg/kg
B= parasitized mice (standard drug group)	Artesunate	4mg/kg
C= parasitized mice (negative control group)	Distilled H_20	0.2 ml
D= parasitized mice (vehicle control group)	Tween 80	0.2 ml
E= parasitized mice (test group 1)	Artesunate + Chloroquine	4mg/kg/25mg/kg
F= parasitized mice (test group 2)	Artesunate + Selenium	4mg/kg/1mg/kg
G= parasitized mice (test group 3)	Artesunate + Zinc	4mg/kg/100mg/kg
H= parasitized mice (test group 4)	Artesunate + vitamin A	4mg/kg/60mg/kg
I= parasitized mice (test group 5)	Artesunate + vitamin E	4mg/kg/100mg/kg

(0.2 ml), group D animals were dosed orally with tween 80 (0.2ml), group E animals received artesunate (4mg/kg) + chloroquine (25mg/kg), group F animals were administered oral doses of artesunate (4mg/kg) and selenium (1mg/kg), group G animals were dosed with artesunate (4mg/kg) and zinc (100mg/kg), group H animals were dosed with artesunate (4mg/kg) and vitamin A (60mg/kg), while group I animals were dosed with artesunate (4mg/kg) and vitamin E (100mg/kg) daily for 4 days respectively (Table 1b). From day 4 post inoculation, daily thin blood film were made to assess the level of parasitaemia, % chemosuppression, parasite clearance time, recrudescence time and mouse survival time.

RESULTS

As shown in Table 2, synergistic schizonticidal activity was more marked with the vitamin A + E combination therapy (94.52%) when compared with any other micronutrient combination after 4 days treatment of established infection. This was closely followed by the vitamin A + selenium combination (94.43%), though the mean difference in parasitemic levels was significant between groups ($F = 2.59$; $P < 0.05$).

Mouse survival time was significantly prolonged ($p < 0.05$) in all the micronutrient combination groups when compared with the controlled group treated with distilled water.

This was also significant ($F=1359.70$; $P<0.05$) between groups. As shown in Figure 1, the decline in parasitemia was sustained in the vitamin A + E treated group till the 4th day post treatment when compared to the chloroquine treated group. In the other micronutrient combination groups there was a steady decline in parasitemia till the 2nd day post treatment; this was not sustained afterwards. However, peak parasitemia was lower on day 17 post treatment in all the micronutrient combination groups when compared with control.

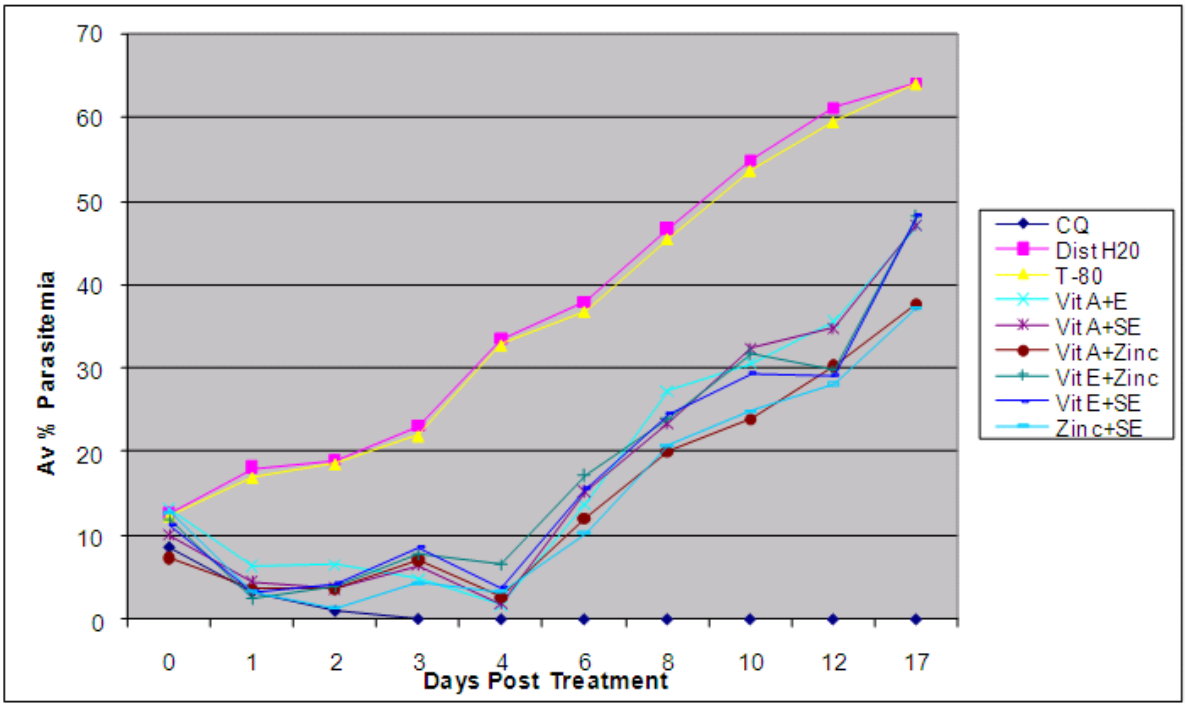
As shown in Table 3, there was a markedly significant chemosuppression in all the micronutrient and standard drug groups when compared with negative control ($P < 0.05$) in the 4 day curative synergistic test using chloroquine resistant ANKA strain. Additionally, parasite clearance was complete in artesunate + zinc and artesunate + selenium group when compared to the artesunate + vitamin A and artesunate + vitamin E group; however, the mean difference

Table 2: Mean Parasitemic Levels of Established *P. berghei* Infection after 4 Days of Treatment (Curative Synergistic Test). n= 5 mice per group.

Groups	Dose (mg/kg)	Av % Parasitaemia	% Suppression	MST (days)
Positive Control (Chloroqiune)	25	0.00 ± 0.00	100	56.00±2.45
NegativeControl (Distilled H_2 0)	0.2ml	3.26 ± 0.94	-	15.80±1.46
Tween-80 (Vehicle)	0.2ml	32.98 ± 1.09	0.84	16.00±1.14
Vitamin A+E	60/100	*1.82 ± 0.10	94.52	*27.80±0.37
Vit A+Selenium	60/1	*1.85 ± 0.43	94.43	*27.20±0.58
Vit A+Zinc	60/100	*2.72 ± 0.79	91.82	*26.50±0.37
Vit E+Zinc	100/100	*6.60 ± 0.85	80.16	*24.40±0.51
Vit E +Selenium	100/1	*3.66 ± 1.48	89.00	*25.60±0.58
Zinc +Selenium	100/1	*3.27 ± 0.19	90.17	*26.20±0.51
ONE WAY ANOVA		F =2. 59 P < 0.05		F=1359.70 P < 0.05

Note: Results are expressed as mean ± SEM. df 4, $p < 0.05$ is significant when compared with control

Figure 1: Parasitaemic Profile of *P. berghei* 17 days Post-treatment in Curative Synergistic Test



in parasitemic level was significant (F = 13.57; P < 0.05). Parasite clearance was more rapid in the artesunate + selenium treated group (2.40±0.40 days) when compared with the artesunate group (3.00±0.32 days), artesunate + chloroquine group (2.60±0.24 days) and artesunate + zinc group (2.60±0.40 days) respectively. Comparatively this was significant P < 0.05 when compared with the artesunate group and artesunate + vitamin A groups and insignificant when compared with the artesunate + chloroquine and artesunate + zinc group respectively. Mean survival time was significantly prolonged in all the treated groups when compared with negative control and vehicle group. Additionally, it was more prolonged in the artesunate + chloroquine group, followed by the artesunate + selenium group. Though the mean difference between the groups was significant (F = 230.71; P < 0.05).

DISCUSSION

It has been observed that mice fed with diet lacking in vitamin E and contains omega-3-fatty acid survive infection with lethal *Plasmodium yoelii* (Taylor et al., 1997). This study was supported by other studies linking vitamin E deficiency with malaria suppression (Levander et al., 1989; Levander and Ager, 1993). However, Maria et al., 2010 suggested that the malaria suppression linked to vitamin E deficiency was primarily due to the disruption in alpha tocopherol transfer protein which acts as an important regulator of vitamin E concentration in the circulation. Additionally, a combination with chloroquine resulted in a markedly significant reduction in parasitemia and an increase in mouse survival rate (Arita et al., 1995; Jishage et al., 2001; Maria et al., 2010). However, these findings are inconclusive. From the study conducted, selenium was found to exhibit a marked chemosuppressive activity against *Plasmodium berghei* infected mice in the 4 day suppressive test and curative test (82.01% and 76.16% respectively). Apart from the study done by Yarrington et al., (1973), no other *in vivo* study has been done till date to corroborate these findings. The work by Yarrington et al., (1973 was inconclusive as it suggested that ducks fed with vitamin E and selenium deficient diet had a more severe manifestation of avian malaria compared with ducks fed with vitamin E and selenium supplemented diet. However, he noted that vitamin E and selenium supplemented diet did not influence the survival time in avian

malaria. This contravenes the finding from the present study which showed a significant increase in mouse survival time when compared with control group treated with distilled water. In a separate experiment he stated that selenium deficient duck were more susceptible to avian malaria (Yarrington et al., (1973). In this same study the author noted that vitamin E and selenium deficiency in swine was associated with a high incidence of microbial infection and that studies in mice fed a diet deficient in factor 3, vitamin E and cystine had diminished natural resistance to Schistosoma mansoni infection. Findings from the present study revealed that selenium, when used as an adjuvant to artesunate and amodiaquine as well as in the presence of other micronutrients demonstrates a remarkably beneficial antimalarial activity. This is supported by; a more rapid parasite clearance demonstrated in the *in vivo* study when used as an adjunct to artesunate in the 4 day curative synergistic test using chloroquine resistant ANKA strain of *Plasmodium berghei.*

However, animal studies on the synergistic antimalarial effects of different antioxidant micronutrient combinations are quite scanty. Results from the present study revealed enhanced antimalarial activity following different micronutrient combination.

Amongst the micronutrient combination groups, vitamin A + E and vitamin A + selenium had a more prominent schizonticidal activity in the 4 day curative test. This was closely followed by the vitamin A + Zinc combination group. This suggests synergism in antimalarial action following antioxidant micronutrient combinations. In addition, mouse survival time was significantly prolonged in the micronutrient combination groups when compared with control ($p<0.05$). Results from other *in vivo* studies are quite scanty. In the present *in vivo* study, parasite clearance was more rapid in the artesunate + zinc group (2.60±0.40 days) when compared with the artesunate group (3.00±0.32 days). Comparatively, the parasite clearance time in the

Table 3: Mean Parasitemic Levels of Established *P. berghei* (chloroquine resistant ANKA strain) Infection after 4 Days of Treatment in Curative Synergistic Test. n= 5 mice per group.

Groups	Dose mg/kg	% Parasitemia	% Suppression	PCT (days)	MST (days)
Chloroquine	25mg/kg	23.23±1.51	60.30	0.00±0.00	25.00±1.22
Artesunate	4mg/kg	0.00±0.00	100	3.00±0.32	53.00±1.84
Distilled H_20	0.2 ml	58.51±3.85	0	0.00±0.00	9.80±0.84
Tween 80	0.2 ml	57.55±3.63	1.64	0.00±0.00	10.20±0.37
Artesunate + Chloroquine	4mg/kg/25mg/kg	*0.00±0.00	100	*2.60±0.24	*55.60±1.34
Artesunate + Selenium	4mg/kg/1mg/kg	*0.00±0.00	100	*2.40±0.40	*54.80±2.34
Artesunate + Zinc	4mg/kg/100mg/kg	*0.00±0.00	100	*2.60±0.40	*53.60±4.62
Artesunate + vitamin A	4mg/kg/60mg/kg	*1.51±0.95	97.42	*3.60±0.87	*51.00±2.92
Artesunate + vitamin E	4mg/kg/100mg/kg	*1.77±1.04	96.97	*2.80±0.37	*52.00±1.64
ONE WAY ANOVA		F = 13.57 P < 0.05		F=13.83 P < 0.05	F = 230.71 P < 0.05

Note: Results are expressed as mean ± SEM. df = 4 *$p< 0.05$ is significant when compared with control

artesunate + chloroquine group (2.60±0.24 days) was not significantly different from the artesunate + zinc group (2.60±0.40 days). Zinc and vitamin A interact in several ways; zinc is a component of retinol binding protein a protein necessary for the transporting of vitamin A in the blood. It is also important in activating the enzyme that converts retinol to retinal (Arif et al., 1987). This is supported by the finding in the present *in vivo* study which showed that zinc and vitamin A combination resulted in a remarkable schizonticidal activity (91.82%) in the 4 day curative synergistic study when compared to other combination groups. This demonstrates the synergism between vitamin A and zinc. These findings are supported by the recent study of Reis et al., (2010) who found that treating mice with a combination of chloroquine and two antioxidant agents, at the first signs of cerebral malaria prevented both inflammatory and vascular damage in the tissues of the brain, as well as the development of persistent cognitive damage in mice. The addition of antioxidants did not diminish the efficacy of chloroquine but rather enhanced its efficacy in eliminating *Plasmodium* from the blood. Similarly combination therapy of antioxidants and artesunate was also effective in treating cerebral malaria and preventing subsequent cognitive impairment in mice. This corroborates the present finding which shows that antioxidant micronutrients have the potential of being used as adjuvants in malaria therapeutics. Thus, providing a low cost effective alternative in the management of malaria.

CONCLUSION

Combination of antioxidant micronutrients (vitamin A, E, selenium and zinc) with standard antimalarials has potential therapeutic benefit in the management of clinical malaria and may be of immense benefit as a therapeutic option when compounded with existing antimalarial formulations as organometallic/organonutritional complexes.

AUTHOR'S CONTRIBUTION

Dr Iribhogbe O.I was responsible for the design, development of the conceptual frame work and execution of the research. Drs Agbaje E.O and Oreagba I.A supervised the work to ensure quality control. Dr Aina O.O and Mr Ota A.D provided technical assistance in the laboratory. Data analysis and manuscript preparation was done by Dr Iribhogbe O.I. All authors proof read and contributed to the intellectual content of the manuscript to ensure quality of presentation.

REFERENCES

1. **Adzu, B., A.K. Haruna, O.A. Salawu, U.D. katsayal and A. Njan**, 2007. *In vivo* antiplasmodial activity of ZS-2A: a fraction from chloroform extract of *Zizyphus spina-christy* root bark against *P. berghei berghei* in mice. *Int. J. Biol. Chem. Sci*. 1(3): 281-286.
2. **Agbaje, E.O. and A.O. Onabanjo,** 1994. Toxicological study of the extracts of antimalarial medicinal plant <I>*Enantia chlorantha*</I>. *Cent. Afr. J. Med;* **40**, 71-3
3. **Arif, A.J., P.D. Mathur, S. Chandra, C. Singh and A.B. Sen,** 1987. Effect of zinc diet on xanthine oxidase activity of liver of mice infected with <I>*Plasmodium berghei*</I>. *Indian J. Malariol;* 24*:*59-63.
4. **Arita, M., Y. Sato, A. Miyata, T. Tanabe and E. Takahashi <I>et al</I>.,** 1995. Human alpha-tocopherol transfer protein: cDNA cloning, expression and chromosomal localization. *Biochem. J*; 306:437–443.
5. **David, A.F., J.R. Philip, L.C. Simon, B. Reto and N. Solomon**, 2004. Antimalarial drug discovery: Efficacy models for compound screening. *Nat. Rev;* 3: 509-520.
6. **Dorsey, D.G., D. Njama, M.R. Kamya, A. Cattamanchi and D. Kyabayinze** *et al*., 2002. Sulfadoxine/pyrimethamine alone or with amodiaquine or artesunate for treatment of uncomplicated malaria: a longitudinal randomised trial. *Lancet*: 360; 2031 -2038.
7. **Schrauzer, G.N**., 2000. Selenomethionine: A Review of Its Nutritional Significance, Metabolism and Toxicity. *J. Nutr;* 130: 1653–1656.

8. **Jishage, K., M. Arita, K. Igarashi, T. Iwata and M. Watanabe** *et al*., 2001. *α*-Tocopherol transfer protein is important for the normal development of placental labyrinthine trophoblasts in mice. *J. Biol. Chem;* 276:1669–72.
9. **Kublin, J.G., J.F. Cortese, E.M. Njunju, R.A. Mukadam and J.J. Wirima <I>et al</I>.,** 2003. Re-emergence of chloroquine-sensitive <I>*Plasmodium falciparum*</I> malaria after cessation of chloroquine use in Malawi. *J. Infect. Dis;* 187:1870 -1875.
10. **Levander, O.A. and A.L. Ager**, 1993. Malarial parasites and antioxidant nutrients. *Parasitology;* 107:S95–106.
11. **Levander, O.A., A.L. Jr. Ager, V.C. Morris and R.G. May**, 1989. Qinghaosu, dietary vitamin E, selenium, and cod-liver oil: effect on the susceptibility of mice to the malarial parasite *Plasmodium yoelii. Am. J. Clin. Nutr;* 50:346–52.
12. **Maria, S.H.**, **Y.U. Yoshiko**, **I. Chie and C. Mayumi**, 2010. Alpha-tocopherol transfer protein disruption confers resistance to malarial infection in mice**.** *Malaria J.,* Vol.**9.**
13. **Obernier, J.A. and R.L. Baldwin**, 2007. Establishing an appropriate period of acclimatization following transportation of laboratory Animals. *ILAR Journal*; 47 (4): 364-369.
14. **Oncu, M., F. Gultekin, E. Kacooz, I. Altuntas and N. Delibas,** 2002. Nephrotoxicity in rats induced by chlorpryfos-ethyl and ameliorating effects of antioxidants. *Hum. Exp. Toxicol;* 21 (4): 223-230.
15. **Oreagba, A.I. and R.B. Ashorobi**, 2006. Evaluation of the antiplasmodial effect of retinol on *Plasmodium berghei* infection in mice. *J. Medical Sci*; 6: 838-842.
16. **Peter, W., H. Portus and L. Robinson**, 1975. The four-day suppressive *in vivo* antimalarial test. *Ann. Trop. Med. Parasitol*; 69:155–171.
17. **Peter, L.T. and V.K. Anatoli**, 1998. The current global malaria situation. Malaria parasite biology, pathogenesis, and protection. ASM press.WDC; pp. 11-22.
18. **Peters, W., R.E. Howells, J. Portus, B.L. Robinson, S. Thomas and D.C. Warhurst**, 1977. The chemotherapy of rodent malaria. XXVII. Studies on mefloquine (WR 142,490). *Ann. Trop. Med. Parasitol;* 71; 407–418.
19. **Reis, P.A., M.C. Clarissa, H. Fernanda, S. Bruno and B. Tatiana et al.,** 2010. Cognitive dysfunction is sustained after rescue therapy in experimental cerebral malaria, and is reduced by additive antioxidant therapy**.** *PLoS Pathog;* 6
20. **Schellenberg, D., E. Kahigwa, C. Drakeley, A. Malende and J. Wigayi et al,** 2002. The safety and efficacy of sulfadoxine-pyrimethamine, amodiaquine, and their combination in the treatment of uncomplicated. *Plasmodium falciparum* malaria. *Am. J. Trop. Med. Hyg.* 67**:** 17 -23.
21. **Staedke, S.G., M.R. Kamya, G. Dorsey, A. Gasasira, G. Ndeezi, E.D. Charlebois and P.J. Rosenthal,** 2001. Amodiaquine, sulfadoxine/pyrimethamine, and combination therapy for treatment of uncomplicated *falciparum* malaria in Kampala, Uganda: a randomised trial. *Lancet*; 358:368 -374.
22. **Tagboto, S. and S. Townson**, 2001. Antiparasitic properties of medicinal plants and other naturally occurring products. *Adv. Parasitol;* 50; 199 -295.
23. **Taylor, D.W., O.A. Levander, V.R. Krishna, C.B. Evans, V.C. Morris and J.R. Barta**, 1997. Vitamin E–deficient diets enriched with fish oil suppress lethal *Plasmodium yoelii* infections in athymic and scid/bg mice. *Infect. Immun*; 65:197–202.
24. **Tekalign, D., M. Yalemtsehay and A. Abebe**, 2010**.** *In vivo* anti-malarial activities of Clerodendrum myricoides, Dodonea angustifolia and Aloe debrana against Plasmodium berghei. *Ethiop. J. Health Dev*; 24(1):26-29.
25. **World Health Organization**, (2003). Assessment and Monitoring of Antimalarial Efficacy for the Treatment of Uncomplicated *falciparum* Malaria. Geneva: Switzerland.
26. **Yarrington, J.T., C.K. Whitehair and R.M. Corwin,** 1973. Vitamin E–selenium deficiency and its influence on avian malarial infection in the duck. *J Nutr*; 103:231–41.