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Nutritional Modulation of Hematological Indices in Malaria: *In vivo* Study in *Plasmodium berghei* Infected Mice

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Malaria infection has been associated with a decrease in hematological parameters. This is linked to micronutrient deficiency which is associated with increasing severity of infection. The present study involves an *in vivo* evaluation of the role of some selected antioxidant micronutrients in the modulation of hematological profile in malaria infection. Rodent malaria model using *Plasmodium berghei* NK-65 strain (chloroquine sensitive) was used. A 4 day curative test was conducted using 45 mice of either sex with a mean weight of 20.05±0.02 g. Fourty mice were inoculated intraperitoneally with 1×10^7 million *Plasmodium berghei* infected erythrocyte and were administered with 0.2 mL of distilled water, 0.2 mL of vehicle; Tween 80 (control and vehicle group), chloroquine 25 mg kg^{-1} and artesunate 4 mg kg^{-1} (standard drug group), vitamin A 60 mg kg^{-1}, vitamin E 100 mg kg^{-1}, selenium 1 mg kg^{-1}, zinc 100 mg kg^{-1} (test group F, G, H and I, respectively) 72 h post inoculation. Results showed a statistically ($p<0.05$) significant difference in total White Blood Cell count (WBC) in the vitamin A, E, selenium and zinc treated groups when compared with apparently healthy non-inoculated control. Additionally, there was a significant increase in MCHC in the zinc treated group when compared to apparently healthy uninfected control (34.21±0.29 g dL^{-1} versus 31.88±0.63 g dL^{-1}; $p<0.05$). In conclusion, use of antioxidant micronutrients as adjuvant may help improve hematological parameters in malaria induced anemia in addition to modulating cell mediated immune response.

Key words: Immunomodulation, micronutrients, hematological profile, anemia

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INTRODUCTION

Dietary Vitamin A administration in early childhood is known to be associated with decreased risk of morbidity and mortality from diarrheal diseases, measles, HIV and malaria infection. These observed effects are due to the activity of vitamin A on the immune system. This immunomodulatory activity has been described previously in clinical trials. This is correlated with outcome of supplementation with vitamin A (Villamor and Fawzi, 2005). Vitamin A is an important nutrient required for maintaining immune function, playing an important role in humoral antibody responses (Shankar, 2000; Stephension *et al*., 2000; Villamor and Fawzi, 2005). Studies by Spallhoiz *et al.* (1990) showed that injection of selenium into rodents enhance host antibody and complement responses showing immunoprotective role of selenium. Selenium which is present in endogenous antioxidants such as glutathione peroxidase is known to augment host antibody and complement response to immunogens such as tetanus toxoid, typhoid toxin, sheep red blood cells and serum immunoglobulins (Spallhoiz *et al*., 1990). The effect of selenium on immunity has been demonstrated in selenium deficient host which includes dysfunction of neutrophil, impaired natural killer cell activity, impaired activation of NADPH-dependent generation of superoxide by granulocytes and decreased humoral antibody response to sheep RBC etc. Furthermore, substantial evidence exists to show that the immune system is being directly affected by selenium. This is supported by studies showing that selenium injection or supplementation resulted in enhanced immunological response against malaria, increased antibody production by B cell, increased T-cell dependent antibody production and increased lymphocyte glutathione peroxidase activity. Additionally, the stimulatory effect on immunity was not observed by all the researchers. Other studies have revealed that zinc is essential for numerous lymphocyte functions involved in the resistance to malaria infection. This includes the production of Immunoglobulin G, IF-γ and TNF-α which enhances the microbicidal efficiency of macrophages (Shankar and Prasad, 1998). Zinc deficiency in humans has been linked to growth impairment, thymic atrophy, lymphopenia, impaired T and B cell activity, impaired neutrophils chemotaxis, a reduction in thymic activity, interferon-γ concentration and the number of CD4+ lymphocytes involved in immunological response (Shankar and Prasad, 1998). The impairment of cellular and humoral immunological function may increase host susceptibility to *Plasmodium falciparum* infection (Shankar and Prasad, 1998; Good *et al*., 1988, 1998). Dietary Zinc supplementation in developing countries has improved delayed cutaneous hypersensitivity response (Sempertegui *et al*., 1996) and increased CD4+dependent lymphocyte response (Sazawal *et al*., 1998). Additionally, dietary Zinc supplementation has been found to reduce the incidence of diarrheal disease and pneumonia in children (Bhutta *et al*., 1999). It has also been found to be beneficial when used as adjunctive therapy in acute diarrheal disease (Bhutta *et al*., 2000; Dutta *et al*., 2000). The effect of vitamin A on clinical malaria could be due to increased phagocytic activity of infected red cells and decreased pro-inflammatory activity of white blood cells (Serghides and Kain, 2002). Malaria infection has been linked to a decrease in hematological parameters (Kulkarni *et al*., 2003) which have been associated with various factors. This includes lysis of parasitized erythrocytes and specific dyserythropioeitic changes (Cusick *et al*., 2005). In addition, there are other reports of a link between micronutrient deficiency and increasing severity of malaria infection (Villamor *et al*., 2007). The essential research question is, what is the likely effect of these antioxidant micronutrients on hematological parameters which are considered to be important health indices of diagnostic significance in clinical evaluation of the state of health in malaria infection? It is therefore pertinent to investigate the significance of these micronutrients on basic hematological parameters viz a viz their role in malaria therapeutics.

MATERIALS AND METHODS

Materials: Chemicals and equipments: Heparinised capillary tubes, Light Microscope (Olympus, Japan), EDTA bottles, Feeding trochars, Syringes (1 mL, 5 mLs), 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) and Artesunate (Emzor Pharmaceuticals, Nigeria).

Preparation of animals: Fourty five in bred and pure Swiss albino mice of either sex weighing between 18-25 g 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, 2006). Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from the cages.

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 (0.2 mL) 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 5 mL 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; Fidock *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.

Preparation of drugs

Chloroquine: Fifty milligram of powdered chloroquine sulphate was dissolved in 20 mL of distilled water. So that 1 mL will contain 2.5 mg of chloroquine sulphate. Dosage administered to the animals in the standard drug group (A) was 25 mg kg^{-1}. Hence the 0.2 mL of solution administered contained 0.5 mg of chloroquine sulphate.

Artesunate: Four milligram of artesunate powder was dissolved in 10 mL of distilled water. Hence 1 mL of distilled water contained 0.4 mg of artesunate. Dosage administered was 4 mg kg^{-1} equivalent to 0.2 mL of solution.

Vitamin A: Two hundred thousand International Units of vitamin A caplet, which is equivalent to 60 mg of vitamin A, was used to prepare the dose administered (60 mg kg^{-1}). The drug was dissolved in 0.2 mL of Tween 80 used as a vehicle and distilled water in a ratio of 0.2:0.2:9.6. To make up a total volume of 10 mL. The final volume of drug administered was 0.2 mL, which is equivalent to 0.495 mg of vitamin A.

Vitamin E: One hundred milligram of vitamin E caplet was dissolved in 0.2 mL of tween 80 and distilled water in a ratio of 0.2:0.2:9.6 making up a total volume of 10 mL. The dose administered to the animal was 100 mg kg^{-1}. Hence the final volume of drug administered to the animal was 0.2 mL, which is equivalent to 1.6 mg of vitamin E.

Selenium: One milligram of selenium was dissolved in 10 mL of distilled water in its powdered form. A dose of 1 mg kg^{-1} body weight was administered to the animals. The final volume of drug administered was 0.2 mL equivalent to 0.0145 mg of selenium.

Zinc: The dose of zinc administered was 100 mg kg^{-1}. 100 mg of zinc was dissolved in 10 mL of distilled water in its powdered form. Zero point two milliliter of the solution was administered which is equivalent to 1.91 mg of zinc.

Drugs/micronutrient administration: A 4-day curative test was performed using the methods of Peters (1965), Peter *et al*. (1975) and Fidock *et al*. (2004). Mice were grouped into nine groups of 5 each and drug/micronutrient administration was done daily for 4 days as shown in Table 1. Antioxidant micronutrients were administered orally using doses based on LD_{50} values as reported by Schrauzer (2000), Oncu *et al*. (2002) and Oreagba and Ashorobi (2006) while the standard dose of chloroquine and artesunate were used.

At the end of the 4 day curative treatment (day 5 post treatment) blood samples were collected via the auxiliary vein into EDTA specimen bottles for laboratory analysis.

Analysis of hematological parameters: Full blood count was estimated using an automated analyzer (Cell-Dyn®,

Table 1: Dosing schedule of drugs/Micronutrients during the 4 day curative Test

Groups	Drugs/Micronurients	Dosage
A = uninfected mice (control group). Not inoculated, no drugs administered	-	-
B = parasitized mice (negative control group)	Distilled water (mL sec)	0.2
C = parasitized mice (vehicle control group)	Tween 80 (mL sec)	0.2
D = parasitized mice (positive control) treated	Chloroquine sulphate (mg kg^{-1})	25
E = parasitized mice (positive control)	Artesunate (mg kg^{-1})	4
F = parasitized mice (test group 1)	Vitamin A (mg kg^{-1})	60^1
G = parasitized mice (test group 2)	Vitamin E (mg kg^{-1})	100
H = parasitized mice (test group 3)	Selenium (mg kg^{-1})	1
I = parasitized mice (test group 4)	Zinc (mg kg^{-1})	100

Abbott, Santa Clara, California). Differential white blood cell counts were estimated by manual count. This was expressed in percentages.

Data analysis: Statistical analyses of the data were performed using statistical soft ware package SPSS version 17.0. Student's t test and one way ANOVA were used to compare the mean of laboratory data between groups. The statistical significance level was set at 95% confidence interval and p value<0.05 was considered significant.

RESULTS

As shown in Table 2, there was a statistically significant reduction in mean parasitemia in the micronutrient treated groups when compared with control group treated with distilled water. Schizonticidal (78.95%) activity was most marked in the selenium treated group when compared to the other micronutrient groups.

As shown in Table 3 , there is a statistically significant difference in total White Blood Cell count (WBC) in the artesunate, vitamin A, E, selenium and zinc treated groups when compared with apparently healthy non-inoculated control ($p<0.05$). This was also observed in the vitamin A, E, selenium and zinc treated groups when compared to negative control group treated with distilled water ($p<0.05$). Similarly, there was a significant difference in platelet count, differential lymphocyte, monocyte and eosinophil count between groups respectively ($F = 1355.43$; $p<0.05$; $F = 108.81$; $p<0.05$; $F = 6.79$; $p<0.05$; $F = 11.25$; $p<0.05$).

As shown in Table 4 , the red blood cell count is significantly reduced in the chloroquine treated group and significantly increased in the artesunate, vitamin A, E, selenium and zinc treated groups when compared to apparently healthy uninfected control ($p<0.05$). Additionally, a significant reduction in hemoglobin concentration was observed in the negative control group while a significant increase in hemoglobin concentration was noted in the vitamin A, E, selenium and zinc groups respectively ($p<0.05$). Interestingly, there is a significant reduction in MCV and MCH values in all the treatment groups when compared with apparently healthy uninfected control. Furthermore, no significant change was observed in the MCHC values (Table 4) between groups and control except for a significant increase in MCHC in the zinc treated group when compared to apparently healthy uninfected control (34.21 ± 0.29 g dL^{-1} versus 31.88 ± 0.63 g dL^{-1}; $p<0.05$).

Table 2: The % chemosupression in *P. berghei* Parasitized mice after 4 days of Treatment (N = 5 mice per group)

Group	Dose (mg kg^{-1})	Av parasitaemia (%)	Suppression(%)
Control not inoculated/no treatment)	-	-	-
(Negative control (Distilled H_2O)	0.2 mL	24.14±2.59	-
Tween-80	0.2 mL	21.82±3.42	9.61
Positive control (Chloroquine)	25	0.00±0.00	100
Artesunate	4	*0.00±0.00	100
Vitamin A	60	*8.90±2.78	63.13
Vitamin E	100	*11.61±2.78	51.90
Selenium	1	*5.08±1.85	78.95
Zinc	100	*9.89±1.11	59.03
ANOVA	F = 17.88 p<0.05		

Values are expressed as X±SEM. *Mean difference is significant at p<0.05 when compared with negative control

Table 3: WBC and platelet count in *P. berghei* parasitized mice after 4 days of treatment (N = 5 mice per group)

Group	Dose mg kg^{-1}	WBC $mm^{-3}\times10^3$	PLT $mm^{-3}\times10^5$	LYM (%)	NEUT (%)	MO (%)	EO (%)	BASO(%)
Control not (inoculated)	-	4.72±132.15	1.21±3006.66	35.60±1.21	63.20±0.66	1.00±0.32	0.00±0.00	0.00±0.00
Negative Control (Distilled H_2O)	0.2 mL	5.18±168.52	*9.16±7752.42	51.80±1.20	43.80±1.16	2.00±0.45	2.00±0.55	0.00±0.00
Tween-80	0.2 mL	4.98±137.42	*3.32±10734.99!	39.00±0.71	61.00±0.71	1.00±0.63	1.00±0.32	0.00±0.00
Positive control (Chloroquine)	25	4.48±92.74	*4.46±5544.37	39.00±0.71	61.00±0.71	0.00±0.00	0.00±0.00	0.00±0.00
Artesunate	4	*5.59±211.34	1.07±2302.17	55.00±0.89	45.00±0.632	0.00±0.00	0.00±0.00	0.00±0.00
Vitamin A	60	*7.13±139.18!	*8.36±17162.75!	60.00±0.71	40.00±0.71	0.00±0.00	0.00±0.00	0.00±0.00
Vitamin E	100	*7.10±100.48!	*6.23±7756.29!	62.00±0.84	38.00±1.22	0.00±0.00	0.00±0.00	0.00±0.00
Selenium	1	*7.680±345.54!	*9.23±10610.84	68.00±0.84	32.00±0.84	0.00±0.00	0.00±0.00	0.00±0.00
Zinc	100	*6.97±67.68!	*2.73±5124.45!	58.00±1.67	42.00±0.84	0.00±0.00	0.00±0.00	0.00±0.00
ANOVA		F= 46.10 p<0.05	F= 1355.43 p<0.05	F=108.81 p<0.05	F=146.11 p<0.05	F= 6.79 p<0.05	F=11.25 p<0.05	

Values are expressed as X±SEM. *Mean difference is significant at p<0.05 when compared to control (not inoculated). ! Mean difference is significant at p<0.05 when compared to negative control

Table 4: Hematological Profile in *P. berghei* Parasitized Mice after 4 day Curative Test (N = 5 mice per group)

Group	Dose mg kg^{-1}	RBC (/mm^3) ×106	Hb (g dL^{-1})	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
Control (not inoculated)	-	5.52±1.56×10^5	12.52±0.20	38.21±0.29	82.66±0.76	28.96±0.94	31.88±0.63
Negative Control (Distilled H_2O)	0.2 mL	5.13±1.01×10^5	*10.90±0.36	33.68±0.62	*54.30±0.45	*17.03±0.12	31.85±0.25
Tween-80	0.2 mL	5.06±1.03×10^5!	11.39±0.18	34.62±0.84	*50.82±0.43!	*17.49±0.27	32.51±0.50
PositiveControl (Chloroquine)	25	*3.68±5.54×10^4!	12.86±0.22	35.08±0.47!	*47.29±0.57!	*16.83±0.17	33.53±0.30
Artesunate	4	*8.23±1.51×10^5!	13.62±0.25	42.22±0.64!	*51.48±0.58!	*16.96±0.18	32.40±0.56
Vitamin A	60	*8.58±8.66×10^4!	*15.37±0.39	42.29±0.38!	*52.17±0.68	*16.47±0.23	32.92±0.46
Vitamin E	100	*9.41±1.43×10^5!	*15.70±0.26	49.02±0.36!	*51.16±0.48!	*16.22±0.16	31.85±0.35
Selenium	1	*8.48±2.72×10^5!	*15.24±0.421	45.48±0.62!	*51.42±0.37!	*17.20±0.24	31.42±0.68
Zinc	100	*6.79±1.6772×10^5!	12.09±0.21	37.75±0.26	*48.18±0.46!	*27.31±0.17!	*34.21±0.29!
ANOVA		F=179.68 p<0.05	F=38.17 p<0.05	F= 98.36 p<0.05	F=388.23 p<0.05	F=368.96 p<0.05	F=3.72 p<0.05

Values are expressed as X±SEM. *Mean difference is significant at p<0.05 when compared with control (not inoculated). [!]Mean difference is significant at p<0.05 when compared with negative control

DISCUSSION

In a study designed to evaluate the potential benefit of combination therapy involving the use of picroliv (an immunomodulator) and the antimalarial (chloroquine) against chloroquine resistant *Plasmodium yoelii* infection in mice, it was observed that picroliv evoked a T-cell proliferating response as well as antibody producing response with an associated enhancement of the efficacy of chloroquine against murine malaria (Puri *et al*., 1992; Bone, 1995). The significant chemosuppressive antimalarial activity demonstrated by the antioxidant micronutrients in the present study is most probably due to immunomodulation. Selenium has similar immunomodulatory properties with picroliv; hence it is expected to evoke a similar enhancement of antimalarial activity when combined with antimalarials. In the present study, schizonticidal activity was most marked in the selenium treated group (78.95%) when compared to other micronutrient groups (zinc; 59.03%, vitamins A and E; 63.13%, 51.90% respectively). Nutritional status plays an important role in humoral and cell-mediated immune function. Micronutrient deficiencies in HIV-infection impair immune responses. This increases the susceptibility to opportunistic infections, enhance HIV disease progression and mother to child transmission. Systemic immune response to HIV infection in children can also be impaired by maternal micronutrient deficiencies. Vitamin A has an important regulatory role in systemic immune function (Ross and Stephensen, 1996; Semba, 1998). Vitamin A deficiency is known to cause impairment in cytotoxic T lymphocyte activity (Sijtsma *et al*., 1990) and neutrophil dysfunction in animals (Twining *et al*., 1997). Additionally, dietary vitamin A supplementation improves natural killer cell cytotoxic activity in rats (Zhao and Ross, 1995) and increases natural killer cell activity in HIV-infected children (Hussey *et al*., 1996). Antibody responses to tetanus toxoid (Semba *et al*., 1992) and measles vaccines (Coutsoudis *et al*., 1992) are also enhanced by vitamin A supplementation in children. In epidemiologic studies, mega doses of vitamin A supplementation reduced the severity of morbidity associated with infections such as measles (Hussey and Klein, 1990), malaria (Shankar *et al*., 1999) and diarrhea, with increased overall survival observed in children with the infections (Fawzi *et al*., 1993).

Antioxidant vitamins are enhancers of immunological function. Vitamin E deficiency is linked with impairment of cell-mediated immunity, neutrophil phagocytosis and lymphocyte proliferation in human and animal studies (Bendich, 1988). Vitamin E administration has immunostimulatory activity in AIDS-infected mice, including increased IL-2 production and natural killer cell cytotoxicity and a reduced production of inflammatory cytokines such as tumor necrosis factor α and IL-6 (Wang *et al*., 1994, 1995). Administration of mega-dose vitamin E in elderly subjects significantly increased lymphocyte proliferation from mitogen stimulation, IL-2 production and delayed type hypersensitivity response (Meydani *et al*., 1990). Long term vitamin E administration at lower doses also increased delayed type hypersensitivity response and enhanced the antibody response to T cell-dependent vaccines (Meydani *et al*., 1997). Vitamin C deficiency had a negative effect on cellular immune responses in animal studies (Bendich, 1988).

Zinc deficiency also has a negative impact on immunity and increased the risk of infection. This is because of zinc's central role in many aspect of immunological activity (Shankar and Prasad, 1998). Zinc is also necessary for the normal function of neutrophils, natural killer cells, macrophages and for the production and activity of T and B lymphocytes. Clinical trials

involving zinc supplementation in children showed significant reduction in morbidity and mortality from malaria, diarrheal and respiratory infections (Black, 1998). Zinc is also important for normal function of cells which are involved in non-specific immunity, such as neutrophils and natural killer cell. B lymphocyte activity and antibody production, particularly immunoglobulin G, is compromised by zinc deficiency. The macrophage plays a central role in many immunologic functions. This is adversely affected by zinc deficiency which can result in dysregulated intracellular killing, impaired cytokine production and phagocytosis. The effect of zinc on these key immunological mediators is hinged on the numerous role of zinc in enhancing basic cellular functions such as DNA replication, RNA transcription, cell division, multiplication and activation. Apoptosis or programmed cell death is potentiated by zinc deficiency. Zinc also functions as an antioxidant and can stabilize membranes.

Selenium is an essential structural component of the antioxidant enzyme glutathione peroxidase and it has numerous important functions in the maintenance of humoral and cell-mediated immunity (Kiremidjian-Schumacher and Stotzky, 1987). Selenium deficiency inhibits neutrophil function, the cytotoxicity of T lymphocytes and natural killer cells, lymphocyte proliferation in response to mitogens, the DTH response, antibody production and resistance to pathogens (Kiremidjian-Schumacher and Stotzky, 1987). In a small study of selenium depletion and supplementation in patients with gut failure and receiving parenteral nutrition, 2-4 months of supplementation with a moderate dose of selenium improved lymphocyte responses to various mitogens and antigens (Peretz *et al*., 1991).

In the present study, a significant increase in total WBC count was observed in the artesunate, chloroquine, vitamin A, vitamin E, zinc and selenium groups when compared with apparently healthy uninfected control. Although there was a non significant increase in total WBC count in the negative control and vehicle group, this increase is a reflection of the elevation in WBC count associated with malaria infection as reported by Lucien *et al*. (2010). The marked elevation of total WBC count in the antioxidant micronutrient treated group as observed in the present study is a reflection of the additional immunostimulating and immunomodulatory role of the micronutrients in malaria infection. Recent studies among children with *falciparum* malaria revealed that a fairly low lymphocyte and monocyte counts were independently associated with morbidity (Lucien *et al*., 2010). However, in the present study no significant change was observed in lymphocyte and monocyte count in the micronutrient treated groups. This suggests that antioxidant micronutrients may actually be involved in the modulation of lymphocyte and monocyte activity. A platelet count of less than 150×10^3 mm^{-3} of blood was found in 13% of the subjects with *falciparum* malaria in the study of Lucien *et al*. (2010). However, the finding in the present study revealed a markedly significant elevation of absolute platelet count in the micronutrient group. This suggests that antioxidant micronutrient may also be involved in the modulation of platelet activity in malaria. Other studies assessed the hematopoietic radio-protective efficacy of an oral formulation consisting of selenomethionine,α-lipoic acid, N-acetylcysteine, vitamin C and vitamin E succinate in vivo using animal survival and immune cell counts as the end points. These studies demonstrated that orally administered antioxidants were effective in protecting the hematopoietic system from the deleterious effects of ionizing radiation as well as in increasing survival. Other data indicate a highly significant effect of dietary antioxidants in the prevention of neutropenia after low-and high-dose Total Body Irradiation (TBI) (Wan *et al*., 2005, 2006). Dietary antioxidants were also effective in preventing peripheral lymphopenia associated with low-dose TBI but not high-dose TBI (Wambi *et al*., 2008; Wan *et al*., 2005, 2006). These finding corroborate the present study which revealed a significantly elevated WBC and differential count in micronutrient treated group. There was also a remarkable improvement in MCHC values in the zinc treated group as well as a significant increase in hemoglobin concentration in the vitamin A, E, zinc and selenium treated groups. This corroborates earlier findings suggesting an immunomodulating and hemato-protective effect of antioxidant micronutrients in malaria. The malaria parasite *Plasmodium falciparum* is able to adapt to the oxidative stress to which it is exposed during the erythrocytic stages of its lifecycle (Muller, 2004). However, the oxidative stress is partly responsible for the damage of red blood cell that ultimately culminates in decreased hematological parameters in infected individuals. The use of antioxidant micronutrients as adjuvants may be of primary importance in reducing the debilitating effect of depressed hematological parameters resulting from extensive destruction of erythrocyte and other blood cell membrane during malarial infection.

CONCLUSION

Antioxidant micronutrients have immunomodulating and hemato-protective potential hence may be useful as adjunctive therapy in malaria infection.

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