

Effects of vitamin E administration on *Plasmodium berghei* induced pathological changes and oxidative stress in mice

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Abstract. The effects of daily intraperitoneal doses of 1000 i.u/kg body weight of vitamin E on the course of *Plasmodium berghei* NK 65 infection and the parasite-induced anemia as well as alterations in the relative weight of some selected organs and antioxidant status in mice were investigated. The number of parasitized red cells were not initially affected by the vitamin administration but were persistently lowered after 11th day post infection to the termination of the experiment. The *P. berghei* infection was found to induce anemia, significantly ($P<0.05$) increased the relative weight of liver, spleen and kidney but significantly decreased ($P<0.05$) the relative brain weight. However, all the parasite-induced changes in these parameters were significantly ($P<0.05$) ameliorated by the vitamin administration. Furthermore, malonydialdehyde concentration in the serum, liver and brain of infected animals was significantly ($P<0.05$) increased whereas superoxide dismutase and catalase activities were significantly ($P<0.05$) decreased by the infection. But vitamin E administration was found to, a significant degree ($P<0.05$), reversed the disease-induced alterations in these oxidative stress markers. It was concluded that vitamin E at the dose and route used prevented *P. berghei* induced anemia as well as alterations in relative organ weight and antioxidant status in mice.

INTRODUCTION

Malaria infection remains a devastating global health problem with an estimated 300–500 million cases occurring annually leading to 1.5–3 million deaths, mainly children under 5 years old (Rodrigues & Gamboa, 2009). The disease is caused by intracellular protozoan parasites from the genus *Plasmodium* and among the different species, *Plasmodium berghei* (rodent malaria parasite) provides a well established experimental model of malaria infection (Margarida *et al.*, 2006) because it produces a disease thought to closely mimic the features of malaria infection in man (Van der Heyde *et al.*, 2006). An important pathological effects of malaria infections are varying degree of anemia and degenerative changes in some tissues and

organs of infected animals (Becker *et al.*, 2004; Arinola *et al.*, 2005; Guha *et al.*, 2006).

The role of oxidative stress as an important clinical and biochemical mechanism of the disease pathogenesis is increasingly becoming relevant (Postma *et al.*, 1996; Becker *et al.*, 2004; Tjahjani *et al.*, 2008). It results from the high metabolic rate of the rapidly growing and multiplying parasite which produce large quantities of toxic redox active by-products. Central to the generation of oxidative stress is the degradation of host haemoglobin by the parasite. Haemoglobin represents the major source of amino acids for *Plasmodium*, but its degradation in an acidic food vacuole results in the production of toxic free haem and reactive oxygen species (ROS) (Ginsburg & Atamna 1994; Becker *et al.*,

2004). Furthermore, during malaria infections massively recruited and activated monocytes and neutrophils produce increased levels of ROS (Postma *et al.*, 1996). The ROS attack both infected and uninfected erythrocytes membrane polyunsaturated fatty acids and proteins leading to destruction of those cells consequently resulting in anemia (Dondorp *et al.*, 1999; Omodeo-Sale *et al.*, 2003). In addition, neurocognitive injury and neurodegeneration (Reis *et al.*, 2010), cerebral and liver pathological changes (Becker *et al.*, 2004; Guha *et al.*, 2006) and renal failure (Nanda *et al.*, 2004) in *Plasmodium* infections have been associated, at least in part, with the parasite-induced generation of ROS and oxidative stress.

Vitamin E is a powerful antioxidant that acts mainly in the lipid phase of cells and has a primary role in preventing the oxidation of polyunsaturated fatty acids (Eggermont, 2006). It acts via scavenging ROS or their precursors (Yossi *et al.*, 2002). The potentials of antioxidants therapy in malaria treatment has widely been suggested (Postma *et al.*, 1996; Arinola *et al.*, 2005; Herbas *et al.*, 2010) but previous studies were limited to additive or adjunctive effects of the antioxidants. Antioxidant therapy with N acetylcysteine and desferrioxamine as additive to chloroquine treatment was found to prevent the cognitive impairment in cerebral malaria infection (Reis *et al.*, 2010) and the combination of chloroquine with vitamin C was reported to have good potential to reduce *P. berghei* induced oxidative stress without any form of liver toxicity (Iyawe & Onigbinde, 2006). On the contrary, additive therapy with vitamin C did not improve the antimalarial activity of some benzophenone derivatives (Mahajan *et al.*, 2005) whereas co-administration of vitamin E with artesunate was reported to reduce the efficacy of artesunate against malaria infection (Awodele *et al.*, 2007). On the other hand, other authors investigated the role of antioxidants in malaria therapy using hosts' dietary and nutritional manipulation strategies and/or gene knockout studies of crucial gene for antioxidant synthesis or regulation. Herbas & Suzuki (2010) reported

that vitamin C deficiency in a L-gulonolactone oxidase gene knockout mice might not affect the development of malaria parasite in mice whereas inhibition of α -tocopherol transfer protein (α -TTP), a determinant of vitamin E concentration in circulation, confers resistance to malaria infection (Herbas *et al.*, 2010a). It was also reported that α -TTP knockout mice infected with *P. berghei* did not exhibit any pathologic signs of cerebral malaria or alteration in the blood-brain barrier integrity but protection of the blood-brain barrier was lost when dietary supplementation with vitamin E was added to their diet (Herbas *et al.*, 2010b). Thus, most of the previous reports on the effects of antioxidant therapy in malarial infections focused on the additive roles of those antioxidants to some antimalarials or hosts' dietary and nutritional manipulations among others and conflicting results were reported in different studies. Furthermore, some of the antioxidants used in such studies are relatively expensive and not readily available to most of the affected populations.

In order to understand the separate, and perhaps more direct influence of antioxidant therapy to malaria infection, we evaluated the effects of intraperitoneal administration of a powerful lipid phase antioxidant (vitamin E) on *P. berghei* induced anemia, relative organ weight changes and endogenous antioxidant status in mice.

MATERIALS AND METHODS

Experimental Animals

The protocol employed met the rules and regulations governing handling of laboratory animals as stipulated by the animal research ethics committee of Ahmadu Bello University, Zaria (ABUZ), Nigeria. Thirty five male swiss albino mice weighing 20 ± 3 g were obtained from the animal house of Department of Pharmacology, Faculty of Pharmaceutical Sciences, ABUZ, Nigeria. The animals were kept in well ventilated laboratory cages with 12 h day/night cycles and maintained on a ration containing commercial poultry feed (Vital feeds, Jos). Water was also supplied *ad libitum*.

***Plasmodium berghei* and infection of experimental animals**

Blood stage samples of chloroquine sensitive *P. berghei* NK 65 used for this study was obtained from the Protozoology laboratory, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, ABUZ, Nigeria. It was maintained in our laboratory by serial passage in mice. The experimental animals were infected through an intraperitoneal injection of approximately 10^4 infected red cells in cold phosphate buffered saline (Ene *et al.*, 2009).

Treatment of the experimental animals

Thirty five mice were randomly divided into five groups of seven mice each. Mice in three of the groups were each infected, while mice in the other two groups were uninfected. A pair of infected and uninfected groups was treated intraperitoneally with vitamin E (Jinling Pharmaceutical Industries, China) at 1000 i.u/kg whereas another infected group was treated with 25 mg/kg of chloroquine from day 1 post infection to the end of the experiment on day 14 post infection (pi). The remaining pair of infected and uninfected groups was not treated with vitamins.

Samples collection and analyses

Tail blood was collected daily for the estimation of parasitemia (Ene *et al.*, 2009) using the Giemsa-stained thin blood smears technique and packed cell volume (PCV) was determined using the microheamatocrit method at days 0 and 15 pi. The mice were humanely decapitated at day 15 pi, blood collected in plain containers and serum prepared. The serum was stored at -15°C until required. The liver, brain, spleen and kidney samples of the mice were quickly extracted, blotted and weighed. Five hundred milligram each of liver or brain samples was homogenized with 10 ml cold physiological saline. The homogenate was centrifuged at 3000 xg for 20 mins and the supernatant was collected. The extent of lipid peroxidation was determined by measuring the level of the thiobarbituric acid reactive substances formed, expressed as malonyldialdehyde (MDA) concentration (Fraga *et al.*, 1988),

catalase (Aebi, 1984) and superoxide dismutase (SOD) (Misra & Fridovich, 1972) activities were all measured in the serum, liver and brain homogenate samples of all groups of mice.

Statistical analysis: The results are presented as mean $\pm$ standard deviation of seven replicate values. Students' *t*-test was used to compare paired means and a difference was considered statistically significant when $p < 0.05$.

RESULTS

The parasites were first detected in all infected mice on day 3 pi and a statistically similar levels of parasitized red cells were detected in infected untreated and vitamin treated infected groups until day 11 pi where the vitamin treatment begins to lower the parasitemia to the end of the experiment (Figure 1). However, no parasitized red cell was detected in the chloroquine treated group from day 5 pi to the termination of the experiment. All the infected groups developed anemia as indicated by significant ($P < 0.05$) drops in their percentage change in PCV but the anemia observed in the vitamin and chloroquine treated groups was significantly less severe ($P < 0.05$) than the infected untreated group (Table 1).

The *P. berghei* infection was found to significantly ($P < 0.05$) increase the relative weight of liver, spleen and kidney of infected animals which were significantly ($P < 0.05$) ameliorated in the group of infected mice given the vitamin therapy (Table 2). However, the *P. berghei* infection significantly ($P < 0.05$) decreased the relative brain weight which was also restored by the vitamin treatment.

The MDA concentration in the serum, liver and brain significantly ($P < 0.05$) increased while SOD and catalase activities significantly ($P < 0.05$) decreased in the infected untreated group (Table 3). These *P. berghei*-induced anomalies were reversed by the vitamin administration.

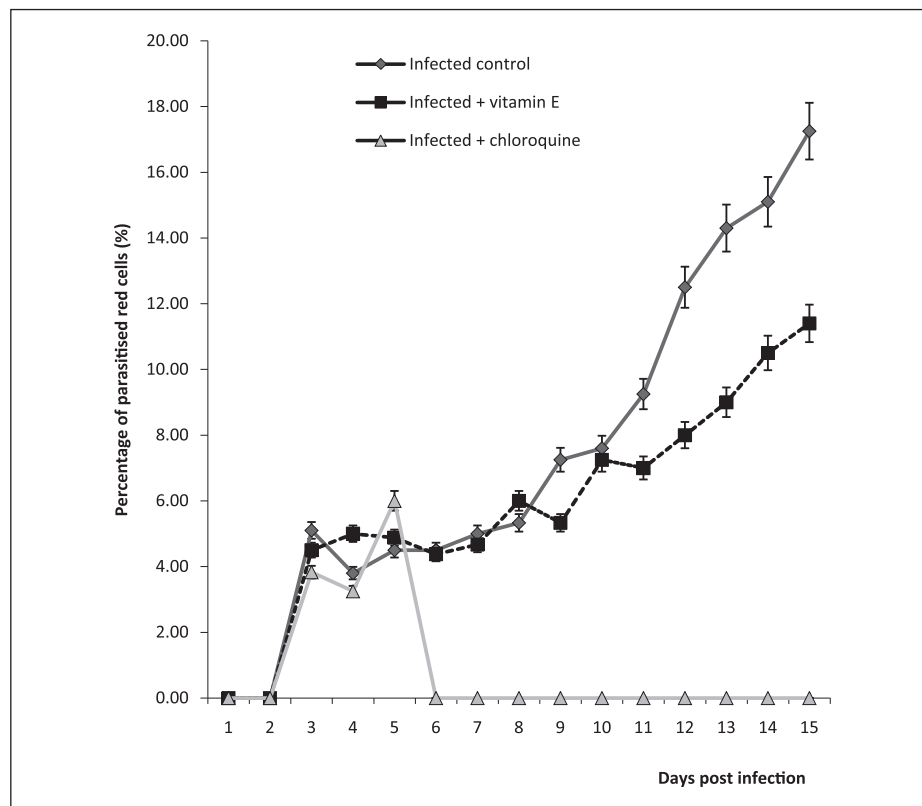


Figure 1. Effect of vitamin E administration on the course of *P. berghei* infection in mice

Table 1. Effects of vitamin E treatment on the PCV levels of *P. berghei* infected mice (n=7)

Parameters	Groups (n=7)				
	Normal control	Vitamin E control	Infected untreated control	Infected treated with vitamin E	Infected treated with chloroquine
Initial PCV (%)	45.20 ± 0.50	44.50 ± 3.32	50.25 ± 4.57	44.75 ± 2.31	49.67 ± 0.58
Final PCV (%)	46.50 ± 1.83	46.25 ± 2.36	32.00 ± 2.00	36.25 ± 1.71	38.00 ± 1.00
*Percentage change in PCV (%)	+4.44 ± 2.23 ^a	+3.87 ± 2.33 ^a	-35.12 ± 11.06 ^b	-18.13 ± 2.31 ^c	-22.19 ± 1.32 ^b

All values are presented as mean ± SD and values with different superscripts letters (a, b, c, d) within a row are statistically different (P<0.05)

*These values represent the percentage differences between initial and terminal PCV values and positive sign (+) indicate increase while negative sign (-) indicate decrease

Table 2. Effects of vitamin E administration on the relative organ weight of *P. berghei* infected mice (n=7)

Groups	Relative organ weight (mg/g)			
	Liver	Spleen	Kidney	Brain
Normal control	52.04 ± 7.22 ^a	6.26 ± 0.94 ^a	12.00 ± 1.09 ^a	20.37 ± 1.32 ^a
Vitamin E control	54.01 ± 4.02 ^a	8.00 ± 0.89 ^b	11.00 ± 1.23 ^a	19.00 ± 1.00 ^a
Infected untreated control	68.00 ± 0.97 ^b	14.09 ± 2.22 ^c	22.00 ± 1.48 ^b	16.05 ± 1.26 ^b
Infected treated with vitamin E	60.00 ± 2.39 ^{a,c}	10.17 ± 1.02 ^d	16.14 ± 1.34 ^c	18.18 ± 1.60 ^{a,b}
Infected treated with chloroquine	68.17 ± 5.68 ^{b,c}	12.00 ± 1.00 ^{c,d}	16.00 ± 1.26 ^c	19.08 ± 2.00 ^{a,b}

All values are presented as mean ± SD and values with different superscripts letters (a, b, c, d) within a column are statistically different (P<0.05)

Table 3. Effects of vitamin E treatment on lipid peroxidation and antioxidant status in the serum, liver and brain of *Plasmodium berghei* infected mice (n=7)

Groups	Lipid peroxidation and antioxidant status							
	Serum			Liver			Brain	
	MDA (μ M)	Superoxide dismutase (U/ml)	Catalase (μ mol/min)	MDA (μ M)	Superoxide dismutase (U/ml)	Catalase (μ mol/min)	MDA (μ M)	Superoxide dismutase (U/ml)
Normal control	73.24 $\pm$ 6.82 ^a	142.97 $\pm$ 20.34 ^a	93.17 $\pm$ 4.19 ^a	33.50 $\pm$ 3.72 ^a	81.10 $\pm$ 5.16 ^a	63.50 $\pm$ 3.69 ^a	22.30 $\pm$ 4.10 ^a	91.23 $\pm$ 12.64 ^a
Vitamin E control	71.62 $\pm$ 8.41 ^a	135.46 $\pm$ 19.67 ^a	90.85 $\pm$ 6.02 ^a	35.16 $\pm$ 5.91 ^a	91.00 $\pm$ 2.93 ^b	60.50 $\pm$ 3.02 ^a	32.74 $\pm$ 7.32 ^a	86.88 $\pm$ 9.30 ^a
Infected untreated control	138.65 $\pm$ 16.95 ^b	63.35 $\pm$ 11.91 ^b	40.27 $\pm$ 7.80 ^b	221.35 $\pm$ 24.45 ^b	59.91 $\pm$ 6.17 ^c	8.25 $\pm$ 2.90 ^b	210.09 $\pm$ 17.39 ^b	64.04 $\pm$ 7.33 ^b
Infected treated with vitamin E	74.54 $\pm$ 6.32 ^a	93.42 $\pm$ 9.05 ^c	55.68 $\pm$ 5.81 ^c	38.09 $\pm$ 10.64 ^a	74.13 $\pm$ 7.84 ^d	48.00 $\pm$ 5.68 ^c	56.82 $\pm$ 8.72 ^c	78.51 $\pm$ 5.01 ^c
Infected treated with chloroquine	97.18 $\pm$ 8.70 ^c	99.25 $\pm$ 11.38 ^c	47.03 $\pm$ 5.26 ^{b,c}	77.25 $\pm$ 12.44 ^c	73.33 $\pm$ 8.42 ^d	42.50 $\pm$ 4.11 ^c	80.15 $\pm$ 5.38 ^d	84.27 $\pm$ 4.22 ^c

All values are presented as mean $\pm$ SD and values with different superscripts letters (a, b, c, d) within a column are statistically different (P<0.05)

DISCUSSION

The role of oxidative stress in the pathogenesis of malaria infections has been elucidated and a large body of evidence (Postma *et al.*, 1996; Becker *et al.*, 2004) suggests that antioxidants could potentially be useful in the treatment of malaria infections. However, previous reports on the use of antioxidants in alleviating the *Plasmodium* induced oxidative stress has largely been confined to investigating their adjunctive and/or additive role in therapy. We report herein the separate effects of vitamin E administration on the course of malaria infection and some pathological alterations caused by the disease. The ability of vitamin E administration to reduce the number of parasitized erythrocytes in the last four days of the experiment is presently, not clear. Although the involvement of vitamin E in the genesis of malarial illness is still very controversial because vitamin E deficiency was reported to have both protective and adverse effects in malarial infection (Nussenblatt & Semba, 2002; Herbas *et al.*, 2010a) but our finding tends to support the hypothesis that intraperitoneal administration of vitamin E negatively impacted on the course of *P. berghei* development in mice.

Anemia is a consistent feature of *Plasmodium* infections (Jones *et al.*, 2002) caused by, among other factors, increased lipid peroxidation as a consequence of oxidative damage to the membrane components of erythrocytes (Dondorp *et al.*, 1999; Omodeo-Sale *et al.*, 2003). The amelioration of the *P. berghei* induced anemia by the vitamin may be attributable to its scavenging effects towards the generated ROS and thereby reducing the oxidative attack to which the erythrocytes membranes are exposed to. However, the inability of the vitamins to completely prevent the disease-induced anemia indicated that, perhaps other aetiological factors are involved in the development of anemia during *P. berghei* infection.

Plasmodium berghei infection has been reported to cause hepato- and splenomegalies in infected mice model

(Arinola *et al.*, 2005) and were attributed to increased phagocytosis of infected cells by macrophages and deposition of malarial pigment as well as activation and hyperplasia of the reticuloendothelial system during the disease (Sawonmi, 1996). It is thus possible that the decrease in the severity of the disease induced anemia caused by the vitamin translated into the correction of the *P. berghei* induced hepato- and splenomegalies. Kidney enlargement and acute renal failure have been demonstrated in *P. berghei* infection partly due to the impairment of the micro-circulation by parasitized erythrocytes (Zailani *et al.*, 2009). The ability of this vitamin to reverse the kidney enlargement caused by the infection could suggest possible involvement of ROS in the development of *P. berghei* associated renal impairments. Conversely, significant decrease in relative brain weight was observed in infected untreated mice. However, cerebral damage has been linked to increased lipid peroxidation and generation of conjugated dienes in brain tissues of mice experimentally infected with *P. berghei* (Reis *et al.*, 2010) and this may consequently result to lowered brain weight. The restoration of relative brain weight in the vitamin treated infected mice could therefore result from their scavenging actions on ROS leading to a reduction in the parasite-induced increase in lipid peroxidation; hence prevented the brain weight loss. On a general note, considering liver, spleen, kidney and brain as representative organs in infected animals, these findings suggest that vitamin E is therapeutically beneficial in alleviating most of the organ weight alterations caused by *P. berghei*.

Increase in the levels of MDA in the serum and organs of *P. berghei* infected mice, indicative of lipid peroxidation (Reis *et al.*, 2010) with concomitant depletion in hosts' SOD and catalase activities are important features of this infection (Areekul & Boonme, 1986; Iyawe & Onigbinde, 2006). However, administration of the vitamin invariably eliminated such alterations. It seemed therefore that the vitamin kept the levels of ROS low thereby reducing the extent of *P. berghei*-induced lipid peroxidation and

consequently spare endogenous primary antioxidant enzymes reserves. This would obviously provide greater protection for cell membrane components as well as other susceptible cellular components and hence significantly retarding the *P. berghei* associated organ pathological effects. Although the observed effects of vitamin E in this work could be linked to the antioxidant activity of the vitamin but other potentially antioxidant substances could act through different molecular mechanism. Melatonin secreted by pineal gland act as scavenger of ROS (Allegra *et al.*, 2003) but was reported to promote synchronization of the intra-erythrocytic cycle of the parasite by Ca^{2+} signaling mechanism (Hotta *et al.*, 2000) and leads to a rise in Ca^{2+} concentration in the *Plasmodium* mitochondria (Gazarini & Garcia, 2004) which indicate that melatonin could disrupt the Ca^{2+} signaling system of the parasite.

In conclusion, this study suggest that vitamin E at the administered dose ameliorated the parasite-induced anemia and organ weight alterations possibly through interfering with lipid peroxidation process as well as sparing endogenous primary antioxidant enzymes reserves.

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