

# Effects of *Plasmodium berghei* Infection and Folic Acid Treatment on Biochemical and Antioxidant Indicators in Mice.

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**Abstract:** The effect of *Plasmodium berghei* and folic acid treatments on mice was investigated. The study derives from the need to examine the possible role of folic acid treatment on erythrocyte fragility, packed cell volume (PCV), bilirubin, total protein, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glucose-6-phosphate dehydrogenase (G6PD), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and reduced glutathione (GSH) in serum and some tissues of mice. Three groups of ten mice each categorized as non-parasitized-non-treated (control), parasitized-non-treated (PnT) and parasitized folic acid treated (P+faT) were used in this study. Results collected and analyzed using standard statistical tool revealed that treatment of parasitized mice with chloroquine and folic acid had significant ( $p < 0.05$ ) reductions in erythrocyte fragility, total bilirubin, total protein, serum and kidney malondialdehyde (MDA) concentrations, liver catalase (CAT) and superoxide dismutase (SOD) activities, but increased ( $p < 0.05$ ) packed cell volume (PCV) and serum CAT and SOD activities in comparison to parasitized non treated (PnT) mice, that had all assayed indices against those of control animals. From these observations, we conclude that under parasitized condition folic acid may efficiently reduce oxidative stress. [Nature and Science 2010;8(8):18-21]. (ISSN: 1545-0740).

**Key words:** *Plasmodium berghei*, Folic acid, Antioxidant, Erythrocyte fragility, oxidative stress.

## 1.0 Introduction

Malaria parasites inside erythrocytes exert oxidative stress within parasitized red blood cells. The parasites generate reactive oxygen species (ROS) from which they are protected (Hunt and Stocker, 1990; Potter et al., 2005), through one or more pathways (Deslauriers et al., 1987; Atamma and Ginsburg 1997; Har-El et al., 1993). The formation of ROS by malaria parasites if not checked by the host cytoprotective enzymes and antioxidants could lead to oxidative damage that could contribute to pathophysiology of many diseases (Gora et al., 2006).

The potential toxicity of free radical generated by malaria parasites are counteracted by a large number of cytoprotective enzymes and antioxidants. One of such antioxidant is ascorbic acid (Behrman et al., 2001). In most endemic areas, chloroquine, used to be the first line therapy for malaria (Olanrewaju and Johnson 2001), until the World Health Organization (WHO) succeeded in promoting the combination treatment for malaria infection (Nosten and Brasseur, 2000).

It has been observed that the treatments of malarial infection in most endemic areas are accompanied with vitamin B complex supplementation with folic acid as component. This organic acid has been implicated in cardiovascular diseases because serum folate concentration has been reported to be an important determinant of serum total homocysteine (tHcy) (Morrison, et al., 1996). However, the role folic acid may play as a single intervention agent in other diseases such as malaria infection is not known.

Though, a preliminary study has shown folic acid to possess antioxidant property in respiration induced oxidative stress (Iyawe and Onigbinde, 2006).

This research is therefore designed to assess the specific effect of folic acid in the treatment of *Plasmodium berghei* infection with considerations to selected biochemical and antioxidant indicators. It is hoped that finding(s) from this work, may give an insight by way of extrapolation as to the usefulness or otherwise of folic acid as an intervening factor in malaria infection management.

## Materials and Methods

### 2.1 Animals

Thirty albino male mice of 8 weeks were used in the study. Observation protocols and method used for maintaining ANKA strains of *Plasmodium berghei* in our laboratory has been previously described (Iyawe and Onigbinde, 2009). The animals used in this study, were treated and handled in the most humane manner.

Three groups of ten animals each respectively categorized as control (None parasitized non-treated), parasitized non-treated (PnT) and parasitized but folic acid-treated (P+faT) were used. Feed and water were respectively given freely. Serum used for assay was harvested as previously reported (Iyawe and Onigbinde, 2009).

### 2.2 Drug Preparation and Administration

Twelve millimeters folic acid containing 2.5 mg/5 ml w/v (NAFDAC cert. No:04-4714) manufactured by

Mopson Pharmaceutical Ltd. Lagos, was diluted with equal volume of distilled water. These preparations brought the active component of each drug to 3 mg/ml these were administered intraperitoneally (25 mg/kg b.w.) during infection for three days, after establishing the presence of parasites in mice with Giemsa stain.

### 2.3 Tissue Extracts Preparation and Assays

Kidney, liver and heart tissues of subjects were obtained as previously described (Iyawe and Onigbinde, 2009). Lipid peroxidation, Superoxide dismutase activity, Catalase activity, Assay of glutathione levels, glucose-6-phosphate dehydrogenase activity (G6PD), gamma glutamyltransferase activity (GGT), Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) assay, serum bilirubin (total, conjugated and unconjugated), Total serum proteins, albumin and Globulin concentration and Erythrocyte fragility were respectively determined as previously described (Iyawe and Onigbinde, 2009).

### 2.4 Statistical Analysis

All values are expressed as Mean±SD. Statistical analysis was performed by one-way Analysis of Variance (ANOVA) and individual comparisons of the group mean values were done using Tukey's Multiple Comparison Test, with the help of Graph pad prism 4.0 software. Value of  $p < 0.05$  was considered significant.

## Results

Table I: Effect of *P. berghei* and folic acid treatment on mice haematological indices.

Parameters	Control	PnT	P+faT
Erythrocyte fragility (%)	0.00 ± 0.00a	37.15 ± 0.77b	15.17 ± 0.90c
Packed Cell Volume (%)	42.71 ± 2.17a	26.83 ± 2.33b	36.83 ± 1.76c
Total Bilirubin (mg/dL)	0.16 ± 0.02a	0.76 ± 0.12b	0.31 ± 0.08c
Direct Bilirubin (mg/dL)	0.10 ± 0.03a	0.68 ± 0.12b	0.26 ± 0.08c
Indirect Bilirubin (mg/dL)	0.06 ± 0.02	0.08 ± 0.01	0.05 ± 0.01

Table II: Effect of *P. berghei* and folic treatments on mice plasma proteins and liver function enzymes.

Parameters	Control	PnT	P+faT
Total Protein (g/L)	67.72 ± 4.17a	89.43 ± 6.24b	69.97 ± 2.71a
Albumin (g/L)	36.63 ± 2.15a	39.14 ± 3.50b	38.93 ± 1.90b
Globulin (g/L)	31.09 ± 2.65a	50.29 ± 6.18b	31.04 ± 4.33a
AST Activity (U/L)	32.07 ± 5.41	36.55 ± 4.93	34.81 ± 5.50
ALT Activity (U/L)	28.17 ± 4.93	30.67 ± 2.86	28.36 ± 5.98
GGT Activity (U/L)	12.22 ± 2.34	14.27 ± 2.43	13.62 ± 3.28

Folic acid treatment in parasitized mice significantly ( $p < 0.05$ ) reduced erythrocyte fragility, total bilirubin and increased PCV compared to PnT mice with values still significantly ( $p < 0.05$ ) lower than that of control group (Table I). Folic acid supplementation decreased ( $p < 0.05$ ) total protein and globulin in comparison to PnT while albumin in both PnT and P + FaT groups increased ( $p < 0.05$ ) compared to control (In Table II).

Folic acid treatment of parasitized mice significantly ( $p < 0.05$ ) reduce oxidative stress and increase ( $p < 0.05$ ) the activity of superoxide dismutase (SOD) and catalase (CAT) in comparison to control (Table III). Also, kidney tissue MDA reduced ( $p < 0.05$ ) to near that of control following folic acid supplementation while liver tissue SOD and CAT activity decreased ( $p < 0.05$ ) compared to PnT and control (Table IV).

Table III: Effect of *P. berghei* and folic treatments on MDA, glutathione and antioxidant enzymes of mice.

Parameters	Control	PnT	P+faT
Malondialdehyde (nmole/mL)	3.36 ± 0.71a	6.54 ± 0.44b	5.40 ± 0.91c
Superoxide Dismutase (U/L)	148.72 ± 10.81a	58.18 ± 18.78b	82.43 ± 3.68c
Catalase (U/L)	199.70 ± 0.14a	166.27 ± 5.92b	182.28 ± 8.17c
Glu-6-P Dehydrogenase (U/L)	29.97 ± 0.78	33.55 ± 1.68	31.26 ± 5.87
Reduced Glutathione (ug/mL)	3.83 ± 0.19a	3.48 ± 0.19a	3.34 ± 0.44c

Table IV: Effect of *P. berghei* and folic acid treatments on MDA, glutathione and antioxidant enzymes of some parasitized mice organs.

Parameters	LIVER			KIDNEY		
	Control	PnT	P+faT	Control	PnT	P+faT
MDA (nmole/mg)	0.74 ± 0.14	0.79 ± 0.12	0.61 ± 0.35	0.66 ± 0.10a	0.71 ± 0.12b	0.62 ± 0.08a
SOD (U/L)	57.32 ± 12.53a	66.67 ± 8.26b	41.34 ± 7.63c	41.51 ± 9.70	40.91 ± 3.16	41.34 ± 7.24
Catalase (U/L)	38.57 ± 4.71a	44.16 ± 3.06b	33.54 ± 3.67c	24.66 ± 1.80	23.89 ± 2.47	24.76 ± 2.03
Glu-6-P-D (U/mg)	0.07 ± 0.01a	0.05 ± 0.01b	0.05 ± 0.18b	0.04 ± 0.10	0.03 ± 0.08	0.03 ± 0.10
GSH (ug/mg)	0.69 ± 0.10a	0.58 ± 0.10b	0.57 ± 0.20b	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.02

Legends to Tables: Results are presented as mean + SD of triplicate determinations (n=10). Values in same row with different letters are significantly different (P<0.05).

#### 4.0 Discussions

Malarial anaemia is reported to be complex as it involves red blood cell destruction either by the parasites or as a result of immune response or both (Mulenga *et al.*, 2006).

The reduced erythrocyte fragility, increased packed cell volume (PCV) and reduced bilirubin levels in parasitized mice group administered folic acid treatment in this study suggests positive influence of folic acid in parasitized mice haematological recovery. A previous report had indicated poor dietary intake of folic acid, rapid erythroid hyperplasia as factors that may deplete folate stores which may lead to delayed haematological recovery (Fleming, 1981).

The reason for the observed effect of folic acid in this work may not be separated from the requirement of this molecule in biosynthetic reactions, cell replication and by extensions cell growth and maintenance. It would therefore appear that folic acid under the condition of parasitemia may either support blood forming factors or probably enhance the production of needed DNA required in the formation of red cells, within bone marrow to stimulate erythropoiesis.

The increases in total proteins and globulins in PnT group in comparison to the other two groups could obviously be associated with the presence of malarial parasites in the animals. In a similar study, infection was reported to result in hyperproteinaemia and hyperglobulinaemia in mice and rabbits respectively (Jack and Robert 1965; Orhue *et al.*, 2005).

Interestingly, folic acid treated group had these same indicators reduced. It is important to note that in the

works of Jack (Jack and Robert 1965; Orhue *et al.*, 2005), folic acids were not administered. We therefore rationalise from our observation that parasitemia could induce increased lymphocytes circulation and the activities of these cells by way of phagocytosis may cause reductions in parasite density and consequently a reduction in globulin secretion by lymphocytes. This may have translated to the observed decrease in total serum protein level.

The increased activity in plasma SOD and CAT may be due to the metabolism of phagocytosized red cells by lymphocytes, through the cytochrome P450 pathway mediated by the cytochrome P450 reductase. This enzyme is known to produce superoxide anion that superoxide dismutase converts to hydrogen peroxide, that is utilized by catalase (Richard and Bettie 1997; Jeremy *et al.*, 2002).

Reduction of oxidative stress on parasitized mice treated with folic acid as observed is unique, in that folic acid is not known to act with the membrane bound - tocopherol to scavenge free radicals within the membrane compartment of the cell. It does appear that this may be the very mechanism by which folic acid interact with the lipid bilayer to cause a reduction in membrane lipid peroxidation as compared to PnT mice. The basis for this line of thought derived from the fact that folic acid is capable of sequential double reduction by folate reductase to H<sub>2</sub>F and H<sub>4</sub>F respectively making the molecule become rich in reducing equivalents that may have been used to trap free radicals that would have otherwise be hazardous to the membrane lipid.

The functionality of folic acid in liver and kidney of parasitized mice are seen to effectively control oxidative stress in these organs. The liver SOD and CAT activity respectively reduced compared to PnT and control groups. These reductions in primary antioxidant enzymes may be responsible for the observed non significant ( $p>0.05$ ) increases in MDA concentration in parasitized mice liver tissue. Folic acid treatment appears to be even more effective in kidney tissue as it significantly ( $p<0.05$ ) reduced oxidative stress compared to PnT. This observation draws attention to the possible interaction between kidney cell membrane -tocopheryl radical and folic acid that may have supplied sufficient reducing equivalent to regenerate membrane -tocopherol to control oxidative stress in infected animals.

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