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Ascorbic and Folic Acids Intervention in *P. berghei* Induced Oxidative Stress in Mice

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Research Article

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ABSTRACT

This research attempts to examine the effects of ascorbic and folic acid intervention on the haematology, antioxidants molecules and enzymes of mice exposed to malaria infection. The study involves three groups of control (non-parasitized-nontreated), parasitized-non-treated (PnT) and parasitized ascorbic and folic acid treated (P+as+faT). Intervention with ascorbic and folic acids commenced for three days after parasitemia had been established in mice. Results from this study showed that ascorbic and folic acid intervention in malaria condition reduced ($P<0.05$) total protein, erythrocyte fragility (EF), increased ($P<0.05$) packed cell volume (PCV) in comparison with PnT and control mice groups. Lipid peroxidation product in serum, Superoxide dismutase (SOD) activity and Catalase (CAT) activity and reduced glutathione (GSH) reduced in parasitized mice administered with ascorbic and folic acid doses, as against those of control, whereas SOD activity in Control and CAT activity in PnT observed to increase and decrease, respectively. The extent of lipid peroxidation in kidney was effectively reduced by ascorbic and folic acid compared to PnT. In liver SOD activity, CAT activity, glucose-6-phosphate dehydrogenase (G6PD) activity significantly ($P<0.05$) reduced in P+as+faT as against PnT and control groups. From these observations therefore, we draw the conclusion that ascorbic and folic acids combination in malaria infection may reduce lipid peroxidation and stimulate cellular pathways that enhance the production of high concentrations of hydrogen peroxide.

Keywords: Plasmodium berghei, ascorbic acid, antioxidants, erythrocyte fragility, oxidative-stress;

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1. INTRODUCTION

The mortality and morbidity arising from malaria infections particularly in both tropical and sub-tropical region of the world still remain perturbing. Malaria parasites inside host erythrocytes exert oxidative stress within the parasitized red blood cells. The parasites generate reactive oxygen species (ROS) from which they are protected (Potter et al., 2005).

The ROS generated by the parasites and the mitochondria, an organelle that make ATP by coupling of respiration generated proton gradient, with the proton driven phosphorylation of ADP by F_0F_1 ATPase, are the major intracellular sources of ROS. Therefore oxidative stress in malaria infection may be attributed to both normal and cellular induced generation of ROS (Yau - Huei and Hsin - Chen, 2002).

The formation of ROS particularly by malaria parasites if not checked by the host cytoprotective antioxidants and enzymes could lead to oxidative damage. There are increasing evidences according to Gora et al., (2006), that cellular injuries contribute to pathophysiology of many diseases. Nutritional manipulations of host oxidative stress had been suggested to influence the course of malaria infection (Levender and Ager, 1993).

Nutrients particularly micronutrients have been found to influence host cellular and humoral immunological functions. These essential factors are very important in the body in order for the immune system to cope with the challenges imposed by infectious agents (Asaolu and Igbaakin 2009) including malaria parasites. Ascorbic acid has been shown to be involved in bone formation, folic acid metabolism, formation and maturation of red blood cells and immune response mechanisms (Asaolu and Igbaakin 2009). Antioxidants have been shown to provide protection against oxidative stress induced by malaria (Adelekan et al., 1997) and ascorbic acid had also been shown to be critical in the management of malaria parasite infection (Iyawe and Onigbinde 2009). There is also a report that under parasitized condition folic acid may efficiently reduce oxidative stress in mice (Iyawe and Onigbinde 2010). However, the World Health Organization (WHO) advocates combination therapy as a more effective means of malaria treatment/control strategy particularly in malaria endemic areas. In line with the WHO position on combination therapy particularly for malaria infection, coupled with previous research findings on the individual roles of ascorbic and folic acid in oxidative stress occasioned by *P. Berghei*, we are curious as to the possible effect these agents in combination may play in alleviating oxidative stress induced by malaria parasite infection. This study was therefore designed to specifically examine the role of both ascorbic and folic acid intervention in mice oxidative stress, induced by *Plasmodium berghei*.

2. MATERIALS NAD 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, 2010). The animals used in this study, were treated and handled in the most humane manner. Three groups of ten mice each respectively categorized as control (None parasitized non–treated), parasitized non–treated (PnT) and parasitized, ascorbic and folic acid-treated (P+as+faT) were used. Feed and water were respectively given freely. Sera used for assay were harvested as previously reported (Iyawe and Onigbinde, 2010).

2.2 DRUG PREPARATION AND ADMINISTRATION PROTOCOLS

Ascorbic acid and folic acid were respectively obtained from Emzor Pharmaceutical Industries Ltd., Lagos and Mopson Pharmaceutical Ltd. Lagos. Solutions of ascorbic and folic acids were prepared by diluting 3mL of ascorbic acid mixture (NAFDAC REG NO. 04 – 0262) containing 100mg/5mL with sterile distilled water to a final volume of 60mL. Folic acid solution was prepared by diluting 12mL of folic acid mixture (NAFDAC cert. No: 04–4714) containing 2.5 mg/5 ml w/v was diluted with equal volume of sterile distilled water. These preparations brought the active components in each drug to 3 mg/mL. These drugs were administered intraperitoneally (25 mg/kg b.w.) during infection for three days, after establishing the presence of parasites in mice with Giemsa stain.

2.2.1 TISSUE EXTRACTS PREPARATION AND ASSAYS

Mice liver, kidney and heart were extracted and processed. Organs, haematological and serum assays for lipid peroxidation [Malondialdehyde (MDA)], superoxide dismutase (SOD) activity, catalase activity, assay of reduced glutathione (GSH) 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 albumin and proteins, Globulin concentration and erythrocyte fragility were respectively determined as previously described (Iyawe and Onigbinde, 2010).

2.2.2 STATISTICAL ANALYSIS

Data collected from this study were subjected to one factor analysis of variance (ANOVA) using computer software (InStat, Graphpad Software, SanDiego, CA). $P < 0.05$ was considered significant. LSD was used to determined differences in means at 95% confidence interval.

3. RESULTS AND DISCUSSION

Results indicate that P+as+faT group had its erythrocyte fragility (EF) significantly ($P<0.05$) reduced with increased packed cell volume (PCV) as against that of PnT, with total bilirubin lower ($P<0.05$) in control group in comparison with PnT and P+as+faT groups (Table 1).

Total proteins reduced ($P<0.05$) in P+as+faT group as against an observed increase in that of PnT (Table 2).

Lipid peroxidation reduced in test groups compared to control. Superoxide dismutase (SOD) activity and catalase (CAT) activity were observed to reduce ($P<0.05$) in PnT and P+as+faT compared to control, with higher reductions in SOD of P+as+faT and PnT group for CAT. A Significant ($P<0.05$) decline of reduced glutathione (GSH) was recorded for P+as+faT as against that for control group (Table 3).

In liver tissue SOD and CAT were reduced ($P<0.05$) compared to control and PnT, while Glucose-6-phosphate dehydrogenase (G6PD) and GSH also reduced ($P<0.05$) in comparison with control group. Treatment with ascorbic and folic acids were observed to significantly ($P<0.05$) reduce the level of lipid peroxidation in kidney tissue as against PnT group (Table 4).

Table 1. Effect of *P. berghei, ascorbic* and folic acid on Mice Haematological Indices

Parameters	Control	PnT	P+as+faT
Erythrocyte fragility (%)	0.00 ± 0.00^{c}	37.15 ± 0.77^{a}	16.36 ± 0.77^{b}
Packed Cell Volume (%)	42.71 ± 2.17^{b}	26.83 ± 2.33^{a}	36.87 ± 1.48^{c}
Total Bilirubin (mg/dL)	0.16 ± 0.02^{a}	0.76 ± 0.12^{b}	0.33 ± 0.10^{c}
Direct Bilirubin (mg/dL)	0.10 ± 0.03^{a}	0.68 ± 0.12^{b}	0.26 ± 0.07^{c}
Indirect Bilirubin (mg/dL)	0.06 ± 0.02^{bc}	0.08 ± 0.01^{c}	0.05 ± 0.03^{ab}

PnT (Parasitized non Treated); P+as+faT (parasitized, ascorbic and folic acid-treated).
Data are all in Mean ± SD of triplicate determinations (n=10). Values in same row with different letters are significantly different (P<0.05).

Table 2. Effect of *P. berghei,* ascorbic and folic acid serum proteins

Parameters	Control	PnT	P+as+faT
Total Protein (g/L)	67.72 ± 4.17^{a}	89.43 ± 6.24^{c}	71.63 ± 2.07^{a}
Albumin (g/L)	36.63 ± 2.15^{a}	39.14 ± 3.50^{b}	37.73 ± 2.57^{ab}
Globulin (g/L)	31.09 ± 2.65^{a}	50.29 ± 6.18^{b}	33.90 ± 3.88^{a}
AST Activity (U/L)	32.07 ± 5.41^{a}	36.55 ± 4.93^{a}	34.10 ± 6.59^{a}
ALT Activity (U/L)	28.17 ± 4.93^{a}	30.67 ± 2.86^{a}	29.17 ± 5.46^{a}
GGT Activity (U/L)	12.22 ± 2.34^{a}	14.27 ± 2.43^{a}	13.92 ± 3.21^{a}

PnT (Parasitized non Treated); P+as+faT (parasitized, ascorbic and folic acid-treated).
Data are all in Mean ± SD of triplicate determinations (n=10). Values in same row with different letters are significantly different (P<0.05).

Table 3. Effect of *P. berghei,* ascorbic and folic acid on some Serum Antioxidants in Mice

Parameters	Control	PnT	P+as+faT
Malondialdehyde (nmole/mL)	3.36 ± 0.71^{a}	6.54 ± 0.44^{b}	4.96 ± 0.41^{c}
Superoxide Dismutase (U/L/min)	148.72 ± 10.81^{a}	58.18 ± 18.78^{b}	48.74 ± 6.98^{c}
Catalase (U/L/min)	199.70 ± 0.14^{a}	166.27 ± 5.92^{b}	$178.\ 98 \pm 11.09^{c}$
Glu-6-P Dehydrogenase (U/L)	29.97 ± 0.78^{a}	33.55 ± 1.68^{a}	31.30 ± 5.72^{a}
Reduced Glutathione (ug/mL)	3.83 ± 0.19^{a}	3.48 ± 0.19^{ab}	3.39 ± 0.31^{b}

PnT (Parasitized non Treated); P+as+faT (parasitized, ascorbic and folic acid-treated).
Data are all in Mean ± SD of triplicate determinations (n=10). Values in same row with different letters are significantly different (P<0.05).

Table 4. Effect of *P. berghei*, ascorbic and folic acid treatments on some antioxidant molecules in mice tissues

Parameters	Heart			Liver			Kidney		
	Control	PnT	P+as+ faT	Control	PnT	P+as+ faT	Control	PnT	P+as+ faT
MDA (mole/mg)	0.54 ± 0.17	0.59 ± 0.09	0.57± 0.09	0.74 ± 0.14	0.79 ± 0.12	0.70 ± 0.10	0.66 ± 0.10[a]	0.71 ± 0.12[b]	0.59 ± 0.09[a]
SOD (U/L)	41.51 ± 8.22	41.34 ± 7.63	41.37 ± 7.60	57.32± 12.53[a]	66.67 ± 8.26[b]	41.94 ± 7.10[c]	41.51 ± 9.70	40.91 ± 3.16	41.34 ± 7.43
Catalase (U/L)	24.89 ± 0.75	24.63 ± 0.50	24.64 ± 0.50	38.57 ± 4.71[a]	44.16 ± 3.06[b]	35. 06 ± 4.19[c]	24.66 ± 1.80	23.89 ± 2.47	25.21 ± 2.47
Glu-6-P-D (U/mg)	0.11 ± 0.04	0.08 ± 0.03	0.09 ± 0.03	0.07 ± 0.01[a]	0.05 ± 0.01[b]	0.04 ± 0.13[b]	0.10 ± 0.04	0.80 ± 0.03	0.60 ± 0.03
GSH (ug/mg)	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.69 ± 0.10[a]	0.58 ± 0.10[b]	0.56 ± 0.09[b]	0.37 ± 0.02	0.37 ± 0.02	0.37 ± 0.02

PnT (Parasitized non Treated); P+as+faT (parasitized, ascorbic and folic acid-treated).
Data are all in Mean ± SD of triplicate determinations (n=10). Values in same row with different letters are significantly different (P<0.05).

The reason for assessing the effect of ascorbic and folic acids combination intervention in malaria infection in mice was to ascertain the possible usefulness of such intervention in the management of malaria parasite infections compared to the use of the individual vitamin we had earlier reported (Iyawe and Onigbinde, 2009; Iyawe and Onigbinde, 2010). Ascorbic and folic acid intervention in parasitized mice appears to be beneficial in the management of malaria parasite infection as indicated in Table 1, with significant ($p<0.05$) reduction in erythrocyte fragility and increased mice PCV in contrast to that observed in PnT mice.

Though the values obtained for oxidative stress indicators namely, MDA, SOD, CAT, GSH, Bilirubin, erythrocyte fragility, albumin, G6PD and PCV (Tables 1, 2 & 3) were significantly ($p<0.05$) lower in mice group that received ascorbic and folic acid intervention compared to those of PnT. It may be hypothesized that the low values observed in mice group that received ascorbic and folic acid intervention may be as a result of the quenching effects of these hydrogen rich organic acids on the free radicals generated within the parasitized red blood cell, therefore stabilising the cell membrane from the harmful manifestations of the free radicals generated from parasite degradation of host haemoglobin.

A striking observation in this intervention regime is the significant ($p<0.05$) decrease in SOD and CAT activities compared to PnT and Control groups. SOD is the key defence enzyme against oxidative stress because it has the ability to convert superoxide anion to hydrogen peroxide (Beyer et al., 1989). Catalase is found in blood, bone marrow, mucous membrane, kidney, and liver. It serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. In this way, catalase functions as a scavenger of H_2O_2 formed by SOD and other processes and thereby protecting the cells from the harmful effects of H_2O_2 (David et al., 2001).

With this observation, it would appear that fewer super oxide radicals are produced and used by SOD to generate hydrogen peroxide for catalase in the group that received ascorbic and folic acid.

Susceptibility of mice liver tissue to oxidative stress in malaria infection is seen in the reduction of SOD and CAT activities that act to protect the tissue against oxyradical damage.

It has been reported (Wozen Craft, 1986; Mohan et al., 1992) that oxidative stress is aggravated by a simultaneous reduction in the effectiveness of the antioxidant defence system in red blood cells. This assertion appears to be restricted to red blood cells as reductions in the activity of liver SOD and CAT did not induce significant oxidative stress in liver tissues of mice treated with vitamins.

In a previous report (Agomo et al., 2001) the effect of free radicals and antioxidants in malaria had been investigated using lipid peroxidation product malondialdehyde and glucose–6-phosphate dehydrogenase as indices in Plasmodium falciparum malaria. The conclusion drawn from the study was that there was no correlation between MDA and the presence or deficiency of G6PD. The result presented for kidney tissues in this work appears to affirm the report of Agomo et al. (2001).

4. CONCLUSION

It can be concluded from the data obtained from this study,that the increase in plasma catalase activity is a possible indication that a combined treatment of parasitized mice with

folic and ascorbic acid appears to induce cellular pathways that produces high cellular hydrogen peroxide concentrations, as confirmed by the observed rise in the activity of G6PD in mice that received the vitamin treatment.

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