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Enhanced Antioxidant Capacity Following Selenium Supplemented Antimalarial Therapy in *Plasmodium berghei* Infected Mice

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Abstract. The effect of the co-administration of artemether, lumefantrine and selenium was studied in mice infected with *Plasmodium berghei*parasite. The mice were divided into seven groups of six animals per group.All groups except A were parasitized. Group A (unparasitized/untreated) and B (parasitized/untreated) served as the positive and negative control respectively, these were administered with olive oil. Animals in groups C and D were treated with 8 and 48 mg/kg/bw of artemether and lumefantrine respectively while group E was treated with a combination of artemether and lumefantrine (8: 48 mg/kg/bw). Animals in group F were treated with 0.945 mg/kg/bw of selenium only and group G was treated with a combination of artemether, lumefantrine and selenium (8:48:0.945 mg/kg/bw). All the treatment was done for a three day period. These animals were subsequently anaesthetized and the organs were excised. Homogenates were prepared for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, reduced Glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) assays. The results showed a significant ($p<0.05$) difference in the levels of ALP and MDA in group B, while a significant difference was observed in the levels of ALP, total protein, CAT and MDA in group G when compared with the parasitized group. Histopathological analysis showed no presence of inflammatory cells in group G when compared with group B. It may be concluded that the combination of artemether, lumefantrine and selenium showed a more potent effect against the parasite than the group treated with artemether and lumefantrine, thus, helps to combat post-infection oxidative stress in susceptible cells.
Keyword: Artesunate, lumenfantrine, selenium, *Plasmodium berghei*, antioxidants, histopathology

INTRODUCTION

Malaria is a vector borne disease that remains a major health concern in the world due to emergence and spread of parasite resistance to established antimalarial drugs [1].Malaria has caused an estimated 0.7-1 million deaths per year [2].Almost half of the world's population is at risk of malaria [3] and majority of the cases (78%) occur in the African region [4]. About 97% of the population in Nigeria are at risk of malaria infection andabout a hundred million malaria cases with over three hundred thousand deaths annually has been estimated in Nigeria [5]. Various attempts to keep the infection at arm's length have tholed a lot of setbacks compounded by the rising number of cases of resistance and cross resistance to the first line anti-malaria drugs [6]. The use of artemether combination therapies (ACT) are now being used to combat the infection. Artemether-lumefantrine combination is a typical ACT which is being used for malaria infection. Artemether-lumefantrine contains 20mg artemether and 120 mg Lumefantrine [7]. It is highly efficient and well-tolerated, and has been recommended by the WHO as first line of treatment against malaria since 2004. Artemether has a

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rapid onset of action and it is quickly eliminated from the plasma. It has a half-life of 2-3 hours [8], while lumefantrine is absorbed and eliminated more slowly from the plasma with a peak concentration of 3-4 hours and a t ½ of approximately 4 days in malaria patients [9]. The oral bioavailability of lumefantrine is highly variable and its co-administration with food increases the bioavailability by 16-fold [9]. It is therefore recommended that the combination be given with a small amount of fat such as in the form of milk [10]. The reasoning behind this combined therapy is that artemether first provides a swift symptomatic alleviation by lowering the amount of parasites present before lumefantrine finally removes any residual parasites. This is assumed to be effective as it minimizes the development of resistance to a crawl because the parasites were never exposed to artemether alone considering its quick elimination. Although, they may be exposed to lumefantrine alone; the chances of simultaneously developing resistance to both drugs is low [11]. The combination of artemether-lumefantrine also decreases the carriage of gametocytes; thus having an impact on transmission of malaria as well as its cure and eradication [12].

Micronutrients (vitamins and essential trace elements) play a crucial role in the maintenance of tissue functions [13]. There is a growing interest in their role in enhancing health by preventing or treatment of disease. Selenium is an essential micronutrient to human health. It is required by humans and animals for the function of a number of selenium-dependent enzymes, also known as selenoproteins. Selenium increases the activity of antioxidant selenoenzymes such as selenium-containing glutathione peroxidases (GPx) which is important in cellular antioxidant defense systems, detoxifying peroxides and hydroperoxides [14]. It also protects the liver from reactive oxygen metabolites[15]. The use of antioxidant therapy against malaria infection poses a potential strategy against the infection. As previous studies on malaria using mouse models have studied the effects of micronutrients on this parasite. Therefore, this study aims to determine the possible biochemical and histopathological effects of the co-administration of artemether, lumefantrine and selenium on malaria parasite using mice model.

MATERIALS AND METHODS

Animals

Forty two male albino mice of Wistar strain purchased from the University of Agriculture, Abeokuta, Ogun state, Nigeria weighing between 11-32g were used for the experiment. The animals were heldin well-ventilated cages at room temperature (28-30°C) and under controlled light cycles (12-hr light: dark). They were fed normal laboratory chow and water *ad libitum.*The experimental animals were handled and used in line with the international guide for the care and use of laboratory animals of the National Institute of Health [16].

Preparation of inoculum of chloroquine sensitive strain of *Plasmodium berghei*

Plasmodium berghei NK 65 strain by serial blood passage from mouse to mouse was used for the study. Donor mouse with an increasing parasitaemia of about 20 -30% which was confirmed by thin and thick blood film microscopy was used. 0.2ml of blood was collected using heparinized capillary tubes from the auxiliary plexus of veins in the donor mouse. The blood wasdiluted with 5ml of Phosphate buffer solution(PBS) pH 7.2 so that each 0.2 ml approximately contain 1 x 10^7 infected red cells [17]. Each animal received inocula of about ten million parasites per kilogram body weight, which is expected to produce a steadily rising infection in mice.

Determination of Parasitemia

Blood smear was taken from the tails of the animals unto microscopic slides and made into thick and thin films on both ends of the slides. This was carried out before and after the administration of the drugs. The blood was fixed with methanol and stained with Giemsa stain. The slides were read under the microscope and the parasite count was determined.

Experimental Design

The mice were divided in to seven groups of six animals each and allowed to acclimatized for two weeks having free access to feed and water before the commencement of the experiment. Group 1 served as unparasitized mice and received olive oil and distilled water only; group 2 comprises the parasitized/untreated mice treated only with olive oil and water; group 3 was administered artemether only (8 mg/kg/bw) with oil as vehicle; group 4 was administered lumefantrine only (48 mg/kg/bw) with oil as vehicle; group 5 was administered artemether and lumefantrine only (8: 48 mg/kg/bw) with oil as vehicle; group 6 was administered selenium only (0.945 mg/kg/bw) with oil as vehicle; group 7 was administered the combination of artemether, lumefantrine and selenium (8 :48 :0.945 mg/kg/bw) with oil as vehicle. The animals were dosed for three days after which they were subsequently anaesthetized and sacrificed. Organs were excised for biochemical and histopathological analysis.

Blood Collection and Preparation of Sample

Following completion of the experiment, blood was collected from the experimentalanimals by cardiac puncture using syringe into lithium heparinised bottles for the determination of serum enzyme levels and other biochemical analyses. Heparinised blood samples were centrifuged at 3000rpm for 15minutes to obtain serum[18] and this was collected into clean bottles by pippeting. The animals were quickly dissected and the liver removed, suspended in ice-cold solution andhomogenized. The homogenates were frozen overnight to ensure maximum release ofthe enzymes [19].

Analysis of Biochemical Parameters

Commercial test kits used for all biochemical parameters measured were obtained from Randox Laboratories, United Kingdom. Standard methods were used to estimate GSH[20]. TBARS was analysed and expressed as the amount of MDA formed[21]. Superoxide dismutase was assayed utilizing the technique of Sun and Zigma,1978 [22] while catalase activity was performed by the methods of Sinha, 1972 [23].

Histopathological Analysis

Small pieces of liver were fixed in 10% buffered neutral formalin and processed for embedding in paraffin[24]. Sections of 5-6µm thickness were stained with hematoxylin and eosin, examined for histopathological changes under a compound microscope.

Statistical Analysis

The difference among experimental and control groups were determined using SPSS for Windows XP software programme (version 13.0). Group comparison was done using the analysis of variance (ANOVA) test. Significant difference between control and experimental were assessed by least significant difference (LSD). All data were expressed as mean±SEM; *P*-values less than 0.05 were considered to be significant

RESULTS

There was significant increase in the activity of ALP in the parasitized/untreated group when compared with the unparasitized control group ($p<0.05$). Administration of Artemether only, lumefantrine only, artemether/lumefantrine and the combined (artemether, lumefantine and selenium) group significantly reduced ($P <0. .05$) serum ALP activities of the animals as compared with the positive control group (parasitized/untreated) (table 1). However, only artemether/lumefantrine group significantly reduced the activities of AST as compared with the parasitized group.

TABLE 1. Effects of the co administration of artemether, lumefantrine and selenium on liver function parameters of *Plasmodium berghei* infected mice

GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)	TOTAL PROTEIN (g/l)
Unparasitized/untreated	194.40±4.88	337.20±8.90	53.51±8.12	26.12±3.78
Parasitized/untreated	198.50±2.22	362.00±3.46	105.56±32.55	20.90±4.57
Artemether only	195.00±5.00	364.00±0.00	43.22±4.68[b]	21.12±1.14
Lumefantrine only	194.50±0.96	343.50±15.22	37.67±11.05[b]	25.87± 1.55
Artemether/lumefantrine	193.50±4.12	281.00±38.31[ab]	55.55±10.22[b]	27.13±1.62
Selenium only	192.00±3.65	347.75±15.46	63.37±15.76	24.06±1.90
Artemether, lumefantrine and selenium	191.50±8.66	322.50±14.46	19.03±8.79[b]	17.59±2.25[a]

Values are expressed as mean ± SEM of six replicates. [a]Significant as compared with the control; $p<0.05$.
[b]Significant as compared with the parasitized/untreated group, $p<0.05$

The parasitized group shows a significant ($p<0.05$) increase in the MDA levels when compared with the unparasitized/untreated group. GSH, SOD and CAT levels were all reduced although not significant in the parasitized/untreated group when compared with the unparasitized/untreated group. The co-administration of artemether, lumefantrine and selenium significantly ($p<0.05$) increased the activity of CAT significantly when compared with both the parasitized/untreated group and the unparasitized/untreated group.

TABLE 2. Effects of the co-administration of artemether, lumefantrine and selenium on the antioxidant parameters of *Plasmodium berghei* infected mice

GROUP	GSH (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	MDA (U/mg protein)
Unparasitized/untreated	1.78±0.21	4.81±0.66	31.13±3.50	0.04±0.001
Parasitized/untreated	1.27±0.23	3.67±0.57	29.63±4.13	0.14±0.04[a]
Artemether only	0.91±0.08[a]	4.33±0.26	29.20±8.40	0.09±0.01
Lumefantrine only	0.56±0.05[ab]	2.83±0.19[a]	23.23±2.48	0.08±0.01[b]
Artemether/lumefantrine	1.38±0.11	3.04±0.22[a]	25.32±1.29	0.09±0.005[a]
Selenium only	0.78±0.07[a]	3.33±0.22[a]	27.21±3.22	0.11±0.01[a]
Artemether, lumefantrine and selenium	1.63±0.42	4.69±0.63	44.03±5.91[ab]	0.16±0.02[a]

Values are expressed as mean ± SEM of six replicates. [a]Significant as compared with the control; $p<0.05$. [b]Significant as compared with the parasitized/untreated group, $p<0.05$

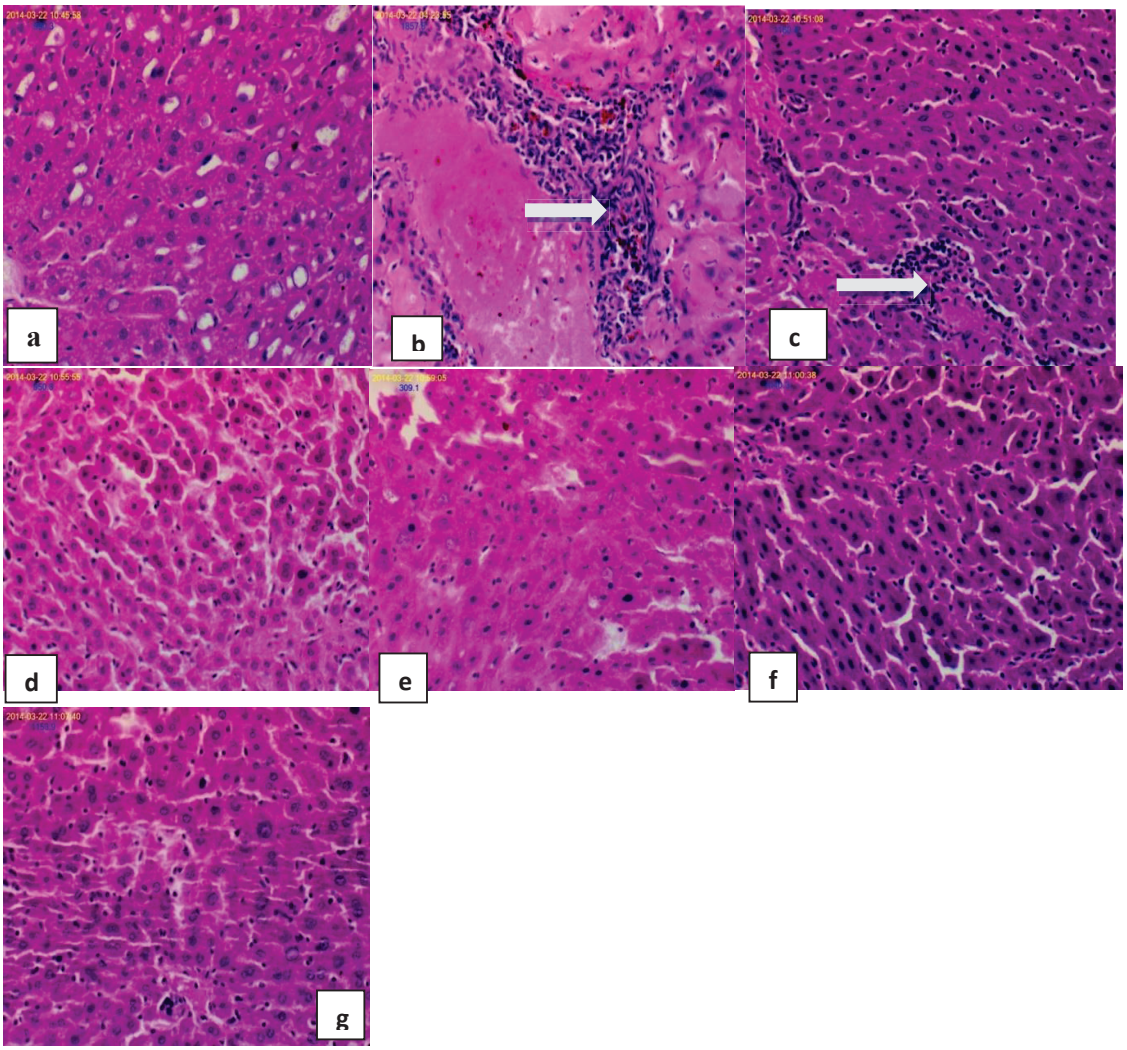

FIGURE 1. Photomicrograph of liver tissues of mice (H&E x160) (a) with normal features of unparasitized mice (b) infected with *P. berghei* (c) treated with 8 mg kg^{-1} bw of artemether(d)treated with 48 mg kg^{-1} bw of lumefantrine(e) treated with 8: 48 mg kg^{-1} bw of artemether and lumefantrine (f)treated with 0.945 mg kg^{-1} bw of selenium (g) co administered with 8: 48: 0.945 mg kg^{-1} bw of artemether, lumefantrine and selenium.

DISCUSSION

The effect of the co-administration of artemether, lumefantrine and selenium on biochemical and antioxidant indices in the Liver of *Plasmodium berghei* infested mice was studied.Marker enzymes measured are effective tools in the diagnosis of diseases emerging from drug administration. Our results show that administration of artemether, lumefantrine and selenium separately seems not to affect the overall protein contents of the mice but their co administration has a significant reduction in total protein content when compared with the unparasitized/untreated group(Table 1). However, the parasitized/untreated group had a non-significant ($p<0.05$) decrease in the levels of total protein when compared with the unparasitized group. The reduction could be as a result of haemolysis and phagocytosis of erythrocytes and other non-infected cells[25]. Based on the work done by Iyawe and Onigbinde [26], parasitemia could induce increased lymphocytes circulation and the activities of these lymphocytes by phagocytosis may cause reductions in parasite density and consequently a reduction in globulin secretion by lymphocytes. This may have possibly resulted in the observed decrease in total protein level. The above results corroborates with the work of Nasir *et al.* [27], in whichthere was a decrease in protein levels in the parasitized group. The thiobarbituric acid assay is an acceptable means of estimating the extent of lipid peroxidation (LPO) through assay of malondialdehyde (MDA) in biological samples [28]. MDA is a product of lipid peroxidation in the erythrocyte membrane [29]. Oxidative stress has been associated with increased erythrocyte osmotic fragility, concurrently with the elevated levels of thiobarbituric Acid Reacting Substances (TBARS) and MDA which are products of lipid peroxidation. A significant ($p<0.05$) increase in MDA levels was observed in the parasitized/untreated group when compared with the unparasitized group(Table 2). The increase in MDA levels suggests high levels of lipid peroxidation leading to tissue damage and failure of the host's antioxidant defence mechanism to prevent the formation of excess free radicals[30]. This is supported by the findings of Leelaonothan and Pidaran, [31] where they reported accumulation of LPO products in rats treated with chloroquine. This marked increase in MDA level could be as a result of the sensitivity of malaria parasites to oxidative damage and this is demonstrated by the efficacy of some antimalarial drugs that are known to act by generating reactive oxygen species (ROS) when administered clinically or experimentally [32]. Major ACTs has been reported to increase the lipid peroxidation level while decreasing plasma antioxidant levels such as GSH and Vitamin C and b-carotene[32]. Co-administration of artemether, lumefantrine and selenium caused a significant ($p<0.05$) increase in the level of CAT (Table 2). This increase may be due to the metabolism of phagocytisized red cells by lymphocytes, through the cytochrome p450 pathway mediated by the enzyme cytochrome p450 reductase, which is known to produce superoxide anion that superoxide dismutase (SOD) converts to hydrogen peroxide which is utilized by catalase [33]. Reduced GSH level is a measure of cellular redox and non-enzymic antioxidant status of cells in higher animals[34]. Our results showed that administration of the antimalarials lowers the overall redox status in the liver, as implied by a significant decrease in the GSH levels in the liver. This is in line with the findings of Ogunbayo *et al.* [35] that reported significant decrease in GSH levels in the blood of rabbits and retina of rats, respectively, following administration of chloroquine. The observed decrease in GSH levels further confirmed the formation of reactive oxygen species or toxic metabolites from these drugs. ALT is found in very high concentrations in the liver which makes it more reliable as a biomarker for hepatocellular damage [36]. Both AST and ALT are biomarkers for liver function and integrity [37]. These liver enzymes are normally raised in acute hepatotoxicity but tend to decrease with protracted intoxication due to liver damage [37, 38]. In this study, a non-significant ($p<0.05$) increase in ALT and AST levels were observed in the parasitized/untreated group when compared with the unparasitized group(Table 1). AST is abundant in the cardiac muscles, skeletal muscles, kidney and testes; therefore any form of disease state that could affect these extra-hepatic tissues is able of causing an elevation of the AST [36]. There was a non-significant ($p<0.05$) decrease in the levels of AST and ALT in the group treated with the combination of artemether, lumefantrine and selenium almost to the levels of the unparasitized group. The reduction of levels of AST and ALT towards the value of the unparasitized group suggests the regeneration process of the liver after drug administration [27]. The work carried out by Iyawe and Onigbinde, [26], is in correlation with this study as the levels AST and ALT decreased after administering an antimalarial to the treatment groups. The biochemical result obtained here is in line with the histological examination which showed the presence of inflammatory cells in the parasitized untreated group while such cells were absent in the combined treatment group (Fig. 1).In conclusion, these results demonstrated the optimization of the efficacy of the combination therapy suggesting that selenium administration contributed to the efficacy of the antimalarial drugs.

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