

BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF COADMINISTRATION OF AMODIAQUINE, ARTESUNATE, AND SELENIUM ON *PLASMODIUM BERGHEI* INFECTED MICE**ABIODUN HUMPHREY ADEBAYO*, GLORIA NWABUGWU OKENZE, OMOLARA FAITH YAKUBU, MOBOLAJI EMMANUEL ABIKOYE**Department of Biochemistry, College of Science and Technology, Covenant University, PMB 1023, Canaan land, Ota, Ogun State, Nigeria.
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ABSTRACT**Objective:** The effect of coadministering artesunate (ART), amodiaquine (AMO), and selenium were studied on mice induced with *Plasmodium berghei*.**Methods:** The study was conducted using 6 groups of 6 male mice each. Group A constitutes the negative control (unparasitized) while Group B represents the parasite control (parasitized) group. Mice in Groups C, D, E and F, respectively, received 2 mg/kg bw of ART, 6.12 mg/kg bw of AMO, combination of AMO and ART, and 0.945 mg/kg bw of selenium in addition to ART and AMO for 3 days. Thereafter, animals were anesthetized, and the organs were excised. Liver homogenate was prepared and used for analysis of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total protein (TP), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and lipid peroxidation (malondialdehyde).**Results:** The results showed no significant alteration in AST and ALT, but ALP was significantly ($p < 0.05$) increased in Group D. In addition, a significant drop ($p < 0.05$) in GSH and SOD activities and significant ($p < 0.05$) increase in TP was observed in group E. Histopathological studies revealed no degenerative change in the morphology of the hepatocytes of mice in Group F whereas Groups D and E showed mild inflammatory cells.**Conclusion:** Conclusively, the combination of ART-AMO therapy with selenium increases the efficacy and reduces potential toxicity of combined antimalarial drugs.**Keywords:** Artesunate, Amodiaquine, Selenium, *Plasmodium berghei*, Antioxidant, Biochemical, Histopathology.© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2018.v11s3.29963>**INTRODUCTION**

Malaria is still the greatest among the dreaded disease affecting most people in Africa, South America, and Southeast Asia [1]. In 2016, malaria caused an estimated 216 million clinical episodes globally leading to 445,000 deaths. Countries in the World Health Organization (WHO) African region accounted for 91% of all the malaria deaths followed by 6% from the WHO Southeast Asia region [2]. The disease is known to be conveyed through the bite of a female *Anopheles* mosquito carrying the parasite [3]. *Plasmodium falciparum* accounts for the most dreadful form of malaria compared to other species of *Plasmodium* that affect humans [4]. Artemisinin-based combination therapy (ACT) has been declared as the first-hand treatment for malaria by the WHO. Most ACTs are known to act through the generation of free radicals which can damage internal tissue proteins, nucleic acids, and membranes by lipid peroxidation [5-7]. A general and readily available ACT includes artesunate (ART)-amodiaquine (AMO) and artemether-lumefantrine and (AQ+AS) [8]. At present, ART and AMO combination is available as separate tablets in packs of 153 mg base of AMO and 50 mg of ART. However, the drugs neglected diseases initiative recently developed the coformulated tablets. The endorsed dosage is 10 mg base/kg AMO together with 4 mg/kg bw ART daily for 3 days [9]. The combo has demonstrated efficiency especially in areas with above 80% cure rate of AMO monotherapy [10]. Selenium is a vital nutrient occupying a position on the periodic table under group VIA [11]. A host of mammals, birds, bacteria, algae, and fishes needs selenium as a vital micronutrient [12]. It is a crucial and significant component of most metabolic processes such as antioxidant defense systems and immune function [13].

The use of antioxidant supplement as part of the treatment regimen of malaria poses a potential strategy against the disease. Therefore,

the objective of this study is to evaluate the probable effect of coadministering ART, AMO, and selenium on malaria parasite-induced mice models.

METHODS**Reagents and drugs**

All reagents used in the study were obtained from Randox Laboratories (Crumlin, UK) unless otherwise stated. ART, AMO, and selenium were obtained from Josrite Pharmaceuticals Ota, Nigeria.

Experimental animals

Male albino Wistar mice (36) having a weight range between 11 and 32 g were sourced from the University of Agriculture, Abeokuta, Nigeria. The animals were housed in the animal house of Biological Science Department Covenant University Ota, Nigeria, and maintained in 12 h light: 12 h dark. They adapted to the environment for 1 week while receiving food and water *ad libitum*. The handlings of all the experimental animals were done following the approved protocols of the Research Ethics Committee of the Department of Biological Sciences, Covenant University Ota, Nigeria.

Experimental design

The mice were grouped into 6 groups of 6 male mice each. Each mouse in the parasitized groups received standard intraperitoneal inoculum of 1.0×10^7 *Plasmodium berghei* parasites. Animals in Groups A and B make up the negative (unparasitized), and parasite control groups were given vehicle (olive oil) only. Mice in Groups C and D were administered 2 mg/kg bw ART and 6.12 mg/kg bw AMO, respectively. Animals in Group E were administered a combination of AMO and ART while

those in Group F received 0.945 mg/kgbw of selenium in addition to ART and AMO. Treatment lasted for 3 days thereafter the animals were subsequently anesthetized and the organs excised.

Biochemical assays

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were ascertained by adopting the method of Reitman and Frankel [14] while the serum alkaline phosphatase (ALP) activity was deduced through enzymatic colorimetric method by Wright *et al.* [15]. Biuret method was followed in determining the protein concentration [16]. Antioxidant parameters were also deduced following established procedures; reduced glutathione (GSH) [17], malondialdehyde (MDA) [18], superoxide dismutase (SOD) [19], and catalase (CAT) [20].

Histological analysis

Adopting the method of Aliyu *et al.* [21]; a fraction of the liver tissue was embedded in 10% formalin after which they were processed for embedding in paraffin [21]. The slide sections were soiled with eosin and hematoxylin stain and analyzed while taking records of photomicrographs using a light microscope.

Statistical analysis

The Statistical Package for the Social Sciences version 15 was employed to analyze data through one-way analysis of variance and Tukey's test. All data values were expressed as a mean±standard error of mean and values were considered statistically significant at $p < 0.05$.

RESULTS

Table 1 shows the effects of the coadministration of selenium, AMO, and ART on the liver parameters in *P. berghei* infected mice. AST and ALT levels were not significantly altered in all groups. However, ALP activity increased significantly ($p < 0.05$) in mice treated with AMO only. A significant decrease ($p < 0.05$) was observed in the group treated with selenium and the antimalarial therapy. Moreover, protein concentration significantly increased ($p < 0.05$) only in the group treated with ART and AMO.

Table 2 shows the influence of coadministration of selenium, AMO, and ART on antioxidant parameters of *P. berghei* infected mice. No significant alteration ($p > 0.05$) in CAT levels in all treated groups was observed whereas a significant drop ($p < 0.05$) in SOD and GSH activities was discovered in Group E on comparison with the positive control.

Similarly, there was a significant elevation ($p < 0.05$) in the MDA levels in all treated groups on comparison with the control groups.

Histopathological analysis

Histopathological studies of mice infested with the parasite showed the presence of inflamed cells (Fig. 1). The group administered the ART, AMO, and selenium combination showed no degenerative changes in the morphology of the hepatocytes. Group D and E showed mild inflammation.

DISCUSSION

This study presents the influence of the coadministering a known ACT (AMO and ART) and selenium on the biochemical and histopathological indices of *P. berghei* infected mice. From our results, exposure of parasitized mice to the antimalarial drugs appears not to significantly affect the liver parameters (ALT and AST). The distortion of the hepatocyte membrane which has been linked to acute liver damage results in the leakage of cytosolic contents of hepatocytes from its membrane [22]. Our observation is in correlation with the findings of Iyawe and Onigbinde [23] as the liver parameters; AST and ALT were not altered significantly with treatment. ALP activity was significantly higher ($p < 0.05$) in AMO treated mice when compared with the normal control group which may indicate biliary obstruction. However, introducing selenium to the therapy led to a decrease in ALP activity suggesting that selenium may have a protective effect against damage associated with increased ALP. This is in line with the study carried out by Erisir *et al.* [24].

A notable ($p < 0.05$) increase in protein concentration was observed in the group administered AMO and ART. High total protein levels in the blood may indicate damage to internal organs such as liver or kidney due to chronic inflammation [25]. This findings correlate with reported studies by Abolaji *et al.* [26] where they also observed increase in protein levels across all groups administered with the antimalarial drug as compared with the control. Studies have shown that most antimalarial drugs tend to increase lipid peroxidation level while decreasing plasma GSH, Vitamin C, and β -carotene [27,28]. From our results, no significant alteration in CAT level was detected in all treatment groups; however, MDA levels were significantly ($p < 0.05$) increased across all treatment groups as compared with the normal control. The increase in MDA levels could be as a result of the damage

Table 1: Effects of coadministration of selenium, amodiaquine, and artesunate on the liver parameters in *Plasmodium berghei* infected mice

Group	AST (units/L)	ALT (units/L)	ALP (units/L)	Protien (g/l)
Normal control (A)	330.00±10.00	180.67±2.67	40.30±3.21	17.13±2.12
<i>Plasmodium berghei</i> only (B)	343.00±10.00	189.00±1.00	43.22±4.67	27.01±1.13
Artesunate only (C)	368.00±4.62	192.67±5.33	32.41±1.46	15.74±1.93
Amodiaquine only (D)	310.00±10.00	189.33±3.53	64.63±6.63a	18.61±0.95
ART/AMO (E)	364.00±6.00	193.00±5.00	36.36±4.53	49.87±1.90*
ART/AMO/SEL (F)	333.33±12.24	191.33±3.33	25.70±9.92ab	16.95±1.35

Values represent mean±SEM of six replicates, * $p < 0.05$ versus untreated unparasitized, ^b $p < 0.05$ versus untreated parasitized. AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, SEM: Standard error of mean

Table 2: Effects of coadministration of selenium, artesunate, and amodiaquine on antioxidant indices in *Plasmodium berghei* infected mice

Group	GSH (units/mg protein)	SOD (units/mg protein)	CAT (units/mg protein)	MDA (units/mg protein)
Normal control (A)	2.63±0.77	4.20±1.43	25.62±2.44	0.03±0.003
<i>Plasmodium berghei</i> only (B)	0.91±0.08	2.66±0.72	26.00±6.48	0.09±0.013
Artesunate only (C)	1.78±0.23	4.50±0.60	31.22±7.30	0.08±0.013a
Amodiaquine only (D)	1.38±0.10	3.98±0.31	31.01±0.61	0.09±0.004a
ART/AMO (E)	0.83±0.06b	1.62±0.73b	14.95±2.67	0.11±0.014a
ART/AMO/SEL (F)	2.31±0.15a	4.60±0.84	33.21±4.45	0.16±0.014ab

Values represent mean±SEM of six replicates, * $p < 0.05$ versus untreated unparasitized, ^b $p < 0.05$ versus untreated parasitized. GSH: Reduced glutathione, SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde, SEM: Standard error of mean, ART: Artesunate, AMO: Amodiaquine

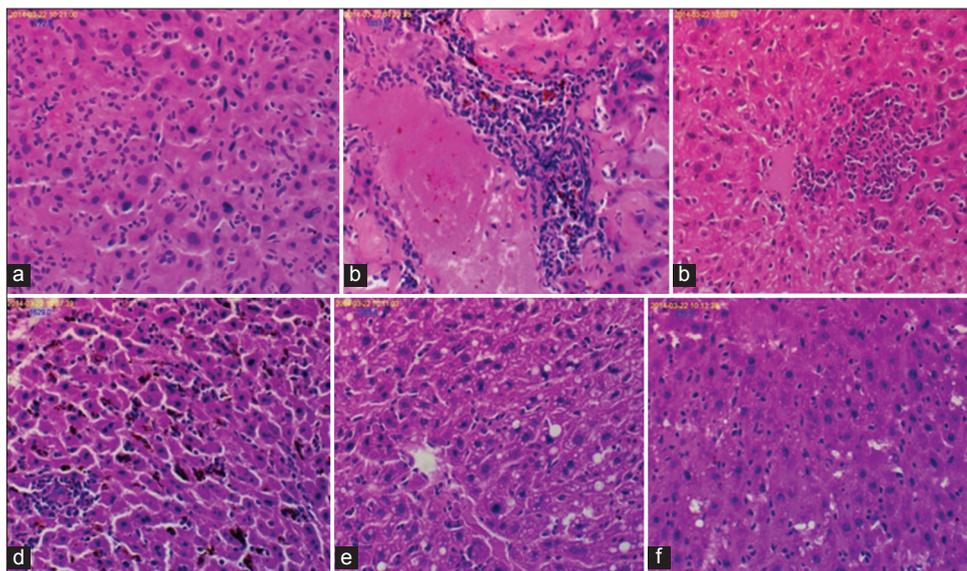


Fig. 1: Photomicrograph of liver of mice: (a) Uninfected showing normal liver (H and E, $\times 160$), (b) infected with *Plasmodium berghei* showing high amounts inflammatory cells (H and E, $\times 160$), (c) treated with 2 mg/kg bw of artesunate (ART) showing no inflammatory cells (H and E, $\times 160$), (d) treated with 6.12 mg/kg bw of amodiaquine (AMO) showing few inflammatory cells (H and E, $\times 160$), (e) liver of mice treated with 2:6.12 mg/kg bw of ART and AMO showing presence of intracytoplasmic vesicles (H and E, $\times 160$), and (f) treated with 2:6.12:0.945 mg/kgbw of ART, AMO and selenium showing a normal liver without inflammatory cells (H and E, $\times 160$)

caused by release of free radicals against cell membranes such as hepatocytes and erythrocytes [29]. However, GSH and SOD were significantly decreased ($p < 0.05$) in ACT treated group as compared with the positive control. This correlates with the findings of other researchers [30,31]. Reduced GSH level signifies the rate of cellular redox and non-enzymatic antioxidant state of cells in higher animals [32]. The observed reduction in GSH level suggests its increased utilization in mopping out the reactive oxygen species generated by the antimalarial drugs. The significant ($p < 0.05$) increase in GSH levels in the selenium-supplemented group signifies the antioxidant potential of the micronutrient in opposing the induction of oxidative stress as a result of ACT administration. This correlates with previous research by Adelekan *et al.* [33] that certain antioxidant micronutrients such as tocopherol could afford defence against malaria-induced oxidative stress and the use of antimalarial drugs. SOD is an antioxidant enzyme that employs superoxide anion as a substrate converting it to hydrogen peroxide which is subsequently utilized by CAT [34]. The significant reduction ($p < 0.05$) of the enzyme in the group treated with ART and AMO could be as a result of SOD mobilization to counteract the oxidative stress produced by malaria and the use of ACT. This is because ACT, an antimalarial containing endoperoxide, tends to generate free radicals to combat malaria parasite, thus reducing the antioxidant enzymes [5]. Koracevic *et al.* [35] reported that the malaria parasite could also degrade these antioxidant enzymes to produce its own protein hence contributing to its depletion in the system. The observed elevation in SOD levels in the group supplemented with selenium further signifies the attempt of the micronutrient to obstruct the induced oxidative stress by ART and AMO administration.

The histopathological analysis of the liver showed the presence of inflammatory cells in the parasitized/untreated group as well as in the group treated with AMO only and the ACT administered group. The presence of these inflammatory cells could be a marker for the degenerative process of the organ as a result of parasite infection. This is also evidenced in the significant increase ($p < 0.05$) in protein concentration in the group treated with ACT when compared to the control. The absence of inflammatory cells in the group coadministered ART, AMO, and selenium suggest the ability of antimalarial drug supplementation with selenium to restore the normal morphology of the liver.

CONCLUSION

This study revealed that the combination of ART-AMO therapy with selenium increases the efficacy and reduces potential toxicity of combined antimalarial drugs.

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