BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF COADMINISTRATION OF AMODIAQUINE, ARTESUNATE, AND SELENIUM ON PLASMODIUM BERGHEI INFECTED MICE

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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 for 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.
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 haematoxylin 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 infected 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 Erissir 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 introducing selenium to the therapy led to a decrease in ALP activity while increasing plasma GSH, Vitamin C, and β-carotene [27,28]. 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 Erissir et al. [24].

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<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (units/L)</th>
<th>AST (units/L)</th>
<th>ALP (units/L)</th>
<th>Proten (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (A)</td>
<td>330.00±10.00</td>
<td>180.67±2.67</td>
<td>4.30±3.23</td>
<td>17.13±2.12</td>
</tr>
<tr>
<td>Plasmodium berghei only (B)</td>
<td>343.00±10.00</td>
<td>189.00±1.00</td>
<td>43.2±4.67</td>
<td>27.01±1.13</td>
</tr>
<tr>
<td>Artemesia only (C)</td>
<td>358.00±4.62</td>
<td>192.67±3.33</td>
<td>32.4±4.16</td>
<td>15.74±1.93</td>
</tr>
<tr>
<td>Amodiaquine only (D)</td>
<td>310.00±10.00</td>
<td>189.33±3.53</td>
<td>50.87±4.09*</td>
<td>16.95±1.35</td>
</tr>
<tr>
<td>ART/AMO only (E)</td>
<td>364.00±6.00</td>
<td>193.00±5.00</td>
<td>56.3±6.63a</td>
<td>18.6±0.95</td>
</tr>
<tr>
<td>ART/AMO/SEL (F)</td>
<td>333.33±12.24</td>
<td>191.33±3.33</td>
<td>36.3±4.53</td>
<td>49.87±1.90</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (units/mg protein)</th>
<th>SOD (units/mg protein)</th>
<th>CAT (units/mg protein)</th>
<th>MDA (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (A)</td>
<td>2.5±0.77</td>
<td>4.20±1.43</td>
<td>25.6±2.44</td>
<td>0.03±0.003</td>
</tr>
<tr>
<td>Plasmodium berghei only (B)</td>
<td>0.9±0.08</td>
<td>2.60±0.72</td>
<td>26.0±6.48</td>
<td>0.09±0.013</td>
</tr>
<tr>
<td>Artemesia only (C)</td>
<td>1.7±0.23</td>
<td>4.50±0.60</td>
<td>31.2±7.30</td>
<td>0.08±0.013</td>
</tr>
<tr>
<td>Amodiaquine only (D)</td>
<td>1.38±0.10</td>
<td>3.98±0.31</td>
<td>31.01±0.61</td>
<td>0.09±0.004</td>
</tr>
<tr>
<td>ART/AMO (E)</td>
<td>0.83±0.06b</td>
<td>1.62±0.73b</td>
<td>14.9±2.67</td>
<td>0.11±0.014</td>
</tr>
<tr>
<td>ART/AMO/SEL (F)</td>
<td>2.3±0.15a</td>
<td>4.60±0.84</td>
<td>33.2±4.45</td>
<td>0.16±0.014ab</td>
</tr>
</tbody>
</table>

Values represent mean±SEM of six replicates, *p<0.05 versus untreated unparasitized, **p<0.05 versus untreated parasitized. GSH: Reduced glutathione, SOD: Superoxide dismutase, CAT: Catalase, MDA: Mlondialdehyde, SEM: Standard error of mean, ART: Artesunate, AMO: Amodiaquine
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 GSH levels in the group supplemented with selenium further 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 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 suggests 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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