Effects of Antioxidant Supplementation on Lipid Profile and Some Hematological Parameters in Malaria Induced Albino Rats.

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Abstract

Oxidative stress is essential in the pathogenesis of malaria and also, the pharmacological basis of most anti-malaria drugs involves the generation of free radicals and its attendant oxidative stress. This study was designed to investigate the effects of antioxidant supplementation on lipid profile and some hematological parameters in malaria induced albino rats. To achieve this, fifteen (36) healthy female adult albino rats weighing between 220-240g were procured and were infected with plasmodium parasite, they all tested positive to malaria parasite, they were later grouped into three (3) experimental groups of twelve (12) animals each. Group A animals received antioxidant foods (carrot, soybeans and oranges) and anti-malaria drugs in addition to their normal rat chow, Group B animals received nutraceuticals (A beta care and Absorbent C) and anti-malaria drugs with their normal rat chow. While Group C animals served as control and were fed with rat chow and anti-malaria drugs respectively for twelve (12) weeks. From the result, there was a significant increase (p<0.05) in the hematological parameters (PCV, WBC, RBC and HGB) of the test groups compared to the control with group A showing more prominence. Also, antioxidant supplementation brings about a significant increase (p<0.05) in the TG and LDL levels of the test groups compared to the control. However, there was a non-significant (p>0.05) decrease in the CHOL and HDL of the test group compared to the control. The reason for these may not be far-fetched given the ability of antioxidants to combat free radicals produced during malaria infection and those produced by anti-malaria drugs.

Keywords: Malaria, serum blood, antioxidant supplementation, lipid profile, haematology

Introduction

Malaria parasite has been reported to be a human pathogen for the entire history of the species (Hayakawa, et al., 2008). Almost all deaths and severe disease transmitted by mosquito are caused by P. falciparum (Talman et al., 2004). Despite the concerted effort by different organization and government, malaria infection still remains one of the most deadly diseases in the world today (Akanbi et al., 2010). The prevalence of malaria infection is higher in the Sahara and sub-tropical region of the world (Akanbi et al., 2010). Malaria transmission can be reduced by preventing mosquito bites with mosquito nets and insect repellant or by spraying insecticides inside houses and draining stagnant water where mosquito laid their eggs (Kilama and Ntoumi, 2009).

Several authors have discussed the implications of free radicals through oxidative stress in the physiopathogenesis of malaria (Pablon, et al., 2002; Huber, et al., 2002; Dondorp, et al., 2003; Omodeo-sale, et al., 2003; Becker, et al., 2004; Narsaria, et al., 2012; Silva, et al., 2011; Yazar, et
al., 2004). This involvement may be related to the pathogenic mechanisms triggered by the parasite (Potter, et al., 2005) as well as free radical production (Keller, et al., 2004) and antioxidant defenses (Sohail, et al., 2007) in host cells to abate the infection. The role of oxidative stress during malaria infection is still unclear. Some authors suggest a protective role, whereas others claim a relation to the physiopathology of the disease (Sohail, et al., 2007). However, recent studies suggest that the generation of reactive oxygen and nitrogen species (ROS and RNS) associated with oxidative stress, plays a crucial role in the development of systemic complications caused by malaria. Malaria infection induces the generation of hydroxyl radicals (OH•) in the liver, which most probably is the main reason for the induction of oxidative stress and apoptosis (Guha, et al., 2006). Additionally, Atamna et al. (Atamna and Ginsburg, 1993) observed that erythrocytes infected with P. falciparum produced OH• radicals and H2O2 about twice as much compared to normal erythrocytes. The existence of oxidative stress and changes in lipid profiles during acute malaria infection has been demonstrated in some studies. This includes depletion of antioxidant, increased plasma lipid peroxidation and altered fluidity of erythrocyte membrane (Das et al., 1993; Sibmooh et al., 2000). Although the oxidative stress appears to be a common phenomenon in acute infection, it may cause a specific consequence in malaria pathogenesis (Sibmooh et al., 2004). Hyperlipidemia, which is one of the indicators of malaria infection could results in depletion of natural antioxidants and facilitate the production of reactive oxygen species which is capable to react with all biological molecules in the body system and exert cytotoxic effects on cellular components (Khovidhunkit et al., 2000; Krishna et al., 2009). Lipoproteins are major lipid component in plasma, and certainly the targets for oxidative stress. It is on this background that this study was designed to assess the effect of antioxidant supplementation on lipid profile and some hematological parameters of malaria induced albino rats.

Materials and Methods

Experimental Animals: A total of 36 female adult albino rats of weighing (220--240) g were obtained from National Veterinary Research Institute (NVRI), Vom near Jos, Plateau state of Nigeria, were used for the study. The animals were randomly assigned into three study groups of 5 animals each. Each study group was individually housed in a Stainless steel cage with plastic bottom grid and a wire screen top. The animal rooms were adequately ventilated and kept at room temperature with a 12-hour natural light-dark cycle. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily. All the animals in the group were fed with Rat chows for first two weeks for acclimatization before commencing the antioxidant vitamins supplement to various groups i.e. group (A and group B, while group C serve as control) for six (12) weeks.

Induction of malaria parasite, anti-malaria drug and antioxidant supplements

The animals in all the groups after acclimatization were induced with malaria Parasite from Nigeria Institute of medical Research Lagos (NIMRI). After one week, blood samples were collected from the animals and they all tested positive to malaria parasite, using blood smear method, after which all the animals were treated for malaria with Chloroquine Sulphate based on the weight of each animal. Immediately after treatment, the animals in Group A received daily
intake of functional foods (carrot, soybeans and oranges) and group B was treated with Drug nutraceutical (Absorbent A and C) based on their body weight, no antioxidants were given to Group C. The antioxidant administration lasted for 6 weeks and was administered orally.

**Preparation of Blood Serum and Assays**

Twenty four hours after the administration of the last dose of dietary supplements on test groups and control respectively, the animals were sacrificed by inhalation of an overdose of chloroform. Blood samples were collected by cardiac puncture into sterilized sample test-tubes, serum samples were then collected into plain container after centrifugation at 3000 rpm for 5 minutes and used for serum lipid analysis.

**Assay of Lipid Profile**

Component lipids were estimated using enzymatic colorimetric diagnostic kits obtained from Randox Laboratories, Antrium, United Kingdom BT 294 QY.

**Total cholesterol estimation**

The estimation of serum cholesterol was done by the end point method of (Allain *et al.*, 1974).

\[
\text{Conc. of cholesterol in sample} = \frac{\Delta A_{\text{sample}} \times \text{conc. of standard}}{\Delta A_{\text{standard}}}
\]

Where \( \Delta = \) absorbance

**Triacylglycerol Estimation (TG)**

The estimation of triglycerides was done by end point method of McGowan *et al.*, (1983).

\[
\text{Triglyceride concentration} = \frac{\text{Absorbance of Sample} \times 200}{\text{Absorbance of Standard}}
\]

**High density lipoprotein Estimation (HDL)**

The estimation of serum HDL was done by precipitation method of Lopez-Virella *et al.* (1977).

\[
\text{HDL-Cholesterol in mg/dl} = \frac{\text{Absorbance of Sample} \times 200}{\text{Absorbance of Standard}}
\]

Factor of 200 was used instead of 50 for calculation due to serum dilution during precipitating step.

**Low density lipoprotein-cholesterol Estimation (LDL-CHOL)**

The estimation of LDL-cholesterol was determined by using formula of Friedwald *et al.* (1972-).
LDL-cholesterol = Total cholesterol – (TG/5 + HDL)

**Assay of hematological parameters:**

Red blood count, total white blood cell count, platelet count, total hemoglobin and packed cell volume (PCV) were determined using fully automated hematology analyzer (Pentra-XL 80, Horbia ABX, USA).

**Statistical analysis**

Data collected were expressed as mean ± standard deviation (SD) and the Student’s T test was used for analysis. Values of P<0.05 were regarded as significant.

**Results and Discussion**

Table 1: Showing the results of the effect of oral administration of antioxidant supplements (functional foods and nutraceutical) on the lipid profile of albino rats.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL-CHOL (mg/dl)</th>
<th>LDL-CHOL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.139±0.02</td>
<td>1.114±0.11</td>
<td>1.191±0.06</td>
<td>2.853±0.04</td>
</tr>
<tr>
<td>B</td>
<td>0.153±0.07</td>
<td>1.823±0.18</td>
<td>1.515±0.09</td>
<td>2.571±0.14</td>
</tr>
<tr>
<td>C</td>
<td>0.262±0.08</td>
<td>1.137±0.53</td>
<td>2.085±0.50</td>
<td>2.470±0.15</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SD. values of (p<0.05) are considered significant.

Table 2: Showing the results of the effect of oral administration of antioxidant supplements (functional foods and nutraceutical) on some hematological parameters of albino rats

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>PCV (%)</th>
<th>WBC (10⁹/L)</th>
<th>HGB (g/l)</th>
<th>RBC (10¹²/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50.98±4.45</td>
<td>7.40±2.07</td>
<td>16.06±1.12</td>
<td>4.98±0.43</td>
</tr>
<tr>
<td>B</td>
<td>47.18±2.96</td>
<td>7.01±1.59</td>
<td>15.90±1.10</td>
<td>4.82±0.50</td>
</tr>
<tr>
<td>C</td>
<td>45.06±4.02</td>
<td>3.40±1.14</td>
<td>13.12±0.87</td>
<td>4.20±0.16</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SD. values of (p<0.05) are considered significant.

Oxidative stress has been implicated in the physiopathogenesis of malaria parasite, through the pathogenic mechanism triggered by the parasite ((Pablon, et al.,2002; Huber, et al.,2002;
Dondorp, et al., 2003; Omodeo-sale, et al., 2003; Becker, et al., 2004; Narsaria, et al., 2012; Silva, et al., 2011; Potter, et al., 2005) as well as free radical production (Keller, et al., 2004) and antioxidant defenses (Sohail, et al., 2007) in the host cell to abate the infection. From the result of this study, there was a significant increase (P<0.05) in the levels of WBC, PCV, HGB and RBC of the test groups (A&B) compared to the control. This may be explained by taking a cursory look at the pattern of malaria pathogenesis. According to (prasannachandra et al., 2006; Granguly et al., 1997, Allison and Enguil, 1983,) malaria pathogenesis involves the invasion of human erythrocytes by the malaria parasite. This brings about metabolic changes in the host cells. The host cells may become vulnerable to damages due to the toxic metabolites derived from both host and parasites. Reactive oxygen species generated in the host parasite interaction cause lyses of erythrocytes and alteration of antioxidants. This is also in line with an independent study carried out by (Ovuakporaye, 2011) who verified the effect of malaria parasite on some hematological parameters where he reported a significant (p<0.05) decrease in the levels of RBC, PCV and hemoglobin due to oxidative stress induced by malaria parasite. It is obvious that the antioxidant supplement fed to the test groups (A&B) abated the free radicals generated by the physiopathogenesis of malaria parasite. This is not surprising giving the potential of antioxidants to combat the free radicals and reverse its attendant oxidative stress as reportedby (Clerc, 1992).

Also, from the result, there was no significant decrease (p>0.05) in cholesterol level of the test groups (A&B) compared to the control (table 1), there was no significant difference (p>0.05) in triglyceride level between test group A compared to the control, but there is a non-significant (p>0.05) increase between test group B (nutraceutical supplement) compared to the control, there was no significant (p>0.05) decrease in the level of HDL of the test groups (A&B) compared to the control, however there is a non-significant (p>0.05) increase in the levels of LDL of the test groups compared to the control, this may also be due to the potential of the antioxidant supplements to abate the free radicals generated during the physiopathogenesis of malaria parasite. Increase in cholesterol, LDL, and Triglyceride levels during malaria infection have been reported to contribute to the pathogenesis of malaria and this could be dangerous to human health as it is capable of causing atherosclerosis if necessary treatment is not adopted. Lipoprotein has been reported to represent a major component of serum needed for the growth of the malaria parasite, (Nilson-Ehle and Nilson-Ehle, 1990). The increases in LDL, cholesterol and triglycerides levels have been reported to be common in malaria positive patients. In this study, the LDL level of the test groups still increased but not significantly (p>0.05) after antioxidant supplementation, meanwhile, there was a non-significant decrease in the levels of cholesterol of the test groups A&B compared to the control

**Conclusion**

Hyperlipidemia and decreased levels of hematological parameters have been reported in malaria infection, this study has demonstrated the ability of antioxidant supplements (functional foods and nutraceutical) to reverse these changes in the lipid profile and hematological parameters of malaria induced albino by combating the free radicals generated by the physiopathogenesis of malaria parasite. Hence antioxidants supplements should also be employed in the treatment of malaria infection.
References


Yazar, S.; Kilic, E.; Saraymen, R.; Ozbilge, H. Serum malondialdehyde levels in patients