Blood Schizonticidal Activity of *Phyllanthus amarus* Enhances Testovarian Antioxidant Defense Capacity in *Plasmodium berghei* Infected Mice

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**ABSTRACT**

In the present study, the crude ethanol leaf extract of *Phyllanthus amarus* was investigated for its in vivo activity against *Plasmodium berghei* malarial parasite in both early and established infections. The study also evaluated the changes in testicular and ovarian antioxidant defense capacity in *P. berghei* infected mice treated with the *P. amarus* leaf extract (100, 200 and 300 mg/kg) using biomarkers (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and malondialdehyde (MDA)) and documental procedures. Results show increased (p < 0.05) ovarian levels of antioxidants (GSH, SOD, CAT and GPx) with insignificant change in MDA of *P. berghei* infected mice when compared with control suggesting good antioxidant capacity. Testes of the *P. berghei* infected mice, however, showed a significant decrease (p<0.05) in the levels of the antioxidant with an associated increase in MDA (p<0.05) amounts when compared with control values. These data indicate that the testes are more susceptible to oxidative stress than the ovaries during malarial infection. Blood schizonticidal activity of *P. amarus* exhibits repository actions against *P. berghei* parasites in a dose-dependent manner as Groups 1, 2, 3 and 4 show established infection values of 0.0, 61.0 ± 8.0, 72.0 ± 6.0 and 87.0 ± 10, respectively. Also, *P. amarus* administration whether in the presence or absence of *P. berghei* infection significantly boosts antioxidant defense capacity and hence invigorated the testes and ovaries as indicated by the empirical data and histopathological evidence. The active phytochemicals, however, need to be identified for further study.

**Keywords:** Testes, ovaries, schizonticidal, *Phyllanthus amarus*, *Plasmodium berghei*.

Introduction

Malaria is an infectious disease caused by a single-celled parasite belonging to the genus *Plasmodium*. There are more than 100 different species but *Plasmodium falciparum* is the deadliest among humans.¹ Malaria threatens the lives of more than one-third of the world’s population and it remains a major killer of human worldwide. The disease exists mainly in the tropical areas of Asia, Africa, and America, where it infects millions of people. Each year, 350 to 500 million cases of malaria occur worldwide² and more than 1 million of its victims, mostly young children and pregnant women, die annually.²,³

It is known that malarial infection is accompanied by increased production of reactive oxygen species (ROS) and the malarial parasites are sensitive to oxidative damage.¹ The univalent reduction of oxygen results in a series of cytotoxic oxygen species such as superoxide anions (O₂⁻¹), hydrogen peroxide (H₂O₂) and hydroxyl radicals (OH)⁻² These highly ROS can cause a wide-spectrum of cell damage including lipid peroxidation, inactivation of enzymes, alteration of intracellular oxidation-reduction state and damage to DNA.⁴,⁵

Mammalian cells, however, possess antioxidant activities which protect the body from the damaging effects of oxygen free radicals.⁴ Although several antioxidant enzymes are essential in this defense system, the most important include glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD). These enzymes act directly on some free radicals, making them less reactive. However, they are not able to act on the highly reactive free radicals such as hydroxyl and peroxynitrite which are chiefly responsible for oxidative and pathological processes. As such, the body uses small molecules that reduce the reactivity of various reactive radicals as an auxiliary antioxidant defense system. This group contains numerous number of molecules, such as vitamins (A, C and E), betacarotene, uric acid and reduced glutathione (GSH) molecule.⁵ A potential source of free radical production in malaria is the host’s haemoglobin molecule. The parasite uses this molecule as a source of amino acids for its own nutrition during the erythrocytic stage of the disease, resulting in the liberation of large amounts of circulating heme. By having Fe²⁺ associated groups, these heme groups are able to induce intracellular oxidative stress, causing changes in erythrocytes and endothelial cells and facilitating the internalization of the parasite in tissues such as the liver and brain.⁶ Although much has been achieved towards the eradication of malaria, it still remains a major source of concern due to compelling circumstances hampering the full control of environmental factors, the mosquito vectors and emerging drug resistance.⁶ Therefore, it is necessary to search for novel and effective anti-malarial agents including those derived from herbs. Herbs have always formed an integral part of human health and so, are used in the treatment of several human diseases. One of such herbal plant is *Phyllanthus amarus*. *P. amarus* is widely used and it has been shown to possess antitumor,¹¹ antiinfective,¹² antimicrobial¹⁴,¹⁵, antileptospiral⁶,¹⁷ and antiparasitological activities in addition to its antimalarial¹⁲ and antioxidant potentials.¹⁸,¹⁹ These observed activities may be due to the

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phytochemical constituents (alkaloids, flavonoids, tannins, saponins, anthroquinones, carbohydrate) already identified in the plant.\textsuperscript{20,21} Thus, the present study attempts to establish a correlation between the effects of antioxidant activities of the plant on its antimalarial activity in the testes and ovaries of \textit{Plasmodium berghei} infected mice treated with \textit{P. amarus} ethanolic leaf extract.

**Materials and Methods**

**Harvesting and preparation of leaf extract**

Fresh plants of \textit{Phyllanthus amarus} were collected from natural habitat in Ozoro, Isoko North LGA of Delta State, Nigeria between February to March 2015. The plant was identified at the Nigeria Institute of Forestry Research, Ibadan, where a voucher specimen (No: FHH109728) has been deposited. The leaves were washed, air-dried and ground into fine powder. The powder (500 g) was soaked in 70\% ethanol overnight and then extracted using a Soxhlet apparatus. The extract was evaporated to dryness under reduced pressure. The percentage yield was 3.6\%. The dried extract obtained was dissolved in distilled water to a concentration of 2.5 mg/mL. The volume administered in millilitre (mL) equivalent to dose (mg/mL) studied was then calculated thus:

\[
V \text{(mL)} = \frac{D \text{(g/kg)} \times P \text{(kg)}}{C \text{(g/mL)}}
\]

\(D\) = dose used (g/kg); \(P\) = body weight (kg); \(C\) = concentration of the extract (g/mL); \(V\) = volume of extract (mL) administered.

**Experimental animals**

Forty-five (45) adult Swiss albino mice of mixed sexes weighing between 20 to 30 g were obtained from the Laboratory Animal Centre, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria and used for the study. Three (3) \textit{Plasmodium berghei} infected (donor) mice were obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria.

**Animal care and handling**

The mice were fed on growers’ mash obtained from Top-Feeds, Sapele, Delta State, Nigeria, and were given clean drinking water ad libitum. The animals were kept in plastic cages, under controlled condition of 12 h light/12 h dark cycle. The animals were maintained in accordance with the guidelines approved by the Animal Ethical Committee, Faculty of Basic Medical Sciences, Delta State University, Abraka.

**Animal grouping, inoculation and extract administration**

The forty-five (45) animals were grouped into nine (\(n = 5/\)group) to allow easy movement and avoid crowding. Group 1 was inoculated with phosphate buffered saline (PBS) (pH 7.4) and treated with placebo-normal saline (normal control); Group 2 was infected with \textit{Plasmodium berghei} (PB) and treated with placebo-normal saline (malaria control); Groups 3, 4 and 5 were parasitized (infected with PB) and treated with 100, 200 and 300 mg/kg b.wt of \textit{P. amarus} ethanolic leaf extract, respectively; Group 6 was also parasitized (infected with PB) but treated with 5 mg/kg b.wt of chloroquine. Group 7, 8 and 9 were inoculated with PBS and treated with 100, 200 and 300 mg/kg b.wt of \textit{P. amarus} ethanolic leaf extract, respectively. The \textit{P. amarus} ethanolic leaf extract and the chloroquine doses were administered as single daily dose using intragastric cannula for a period of seven (7) days. The mice were infected by obtaining parasitized blood (3-4 drops) from the cut tail tip of an infected (donor) mouse. Then, 0.1mL of infected blood was diluted in 0.9mL of phosphate buffer, pH 7.2 and the mice were inoculated with 0.1 mL of the parasitized suspension which contained about ten thousand (10,000) parasites. Parasitaemia was confirmed after 72 hrs using thin blood films prepared from the cut tail tip of the infected mouse and stained with Giemsa stain (1\%), then, viewed with the microscope (Model TH-9845, Serico, China) at x100 magnification as reported by Cheesbrough.\textsuperscript{22}

**Collection of specimen and analysis from experimental animals**

On the 7th day, the experimental animals were fasted overnight and sacrificed under chloroform anaesthetic. Ovaries and testes were collected for biochemical analysis and histopathological examination.

**Histological examination**

Tissues (testes and ovaries) were fixed with Bouin’s solution. They were later sectioned using a microtome and dehydrated in graded alcohol. The dehydrated tissues were embedded in paraffin and thereafter stained with Hematoxylin and Eosin (H and E). The stained tissue slides were mounted under a microscope connected to a computer for views.

**Biochemical analysis**

The testes and ovaries collected were subsequently weighed and twenty percent tissue homogenate prepared using normal saline under cold conditions. The crude tissue homogenate was centrifuged at 2000 rpm for 15 min at 4\(^\circ\)C. The supernatant collected was stored at -20\(^\circ\)C until required for biochemical analysis.

**Antioxidant capacity of specimen**

The ovaries and testes of mice in the various test groups were analyzed to assess the antioxidant capacity of \textit{P. amarus} during malarial infection. Formation of malondialdehyde (MDA) was estimated by determining the accumulation of thiobarbituric acid reactive substances (TBARS)\textsuperscript{32} in the homogenates while the level of reduced glutathione was assessed using the method of Moron et al.\textsuperscript{21} The activities of glutathione peroxide,\textsuperscript{20} superoxide dismutase,\textsuperscript{26} and catalase\textsuperscript{21} were determined using the colorimetric method.

**Statistical analysis**

Experimental data in five replicates were expressed as Mean \pm SD (Standard Deviation). Results were subjected to statistical analysis using one-way analysis of variance (ANOVA) to determine the differences between groups. Differences between means at 5\% level (\(p < 0.05\)) were considered significant.

**Results and Discussion**

The results obtained from this investigation into the schizontocidal activity of \textit{Phyllanthus amarus} and associated changes in testicular and ovarian antioxidant defense capacity in \textit{Plasmodium berghei} infected mice treated with \textit{P. amarus} ethanolic leaf extract show that \textit{P. berghei} malarial parasite infection induced oxidative stress and hence damage in the ovaries (Table 1) and testes (Table 2) of experimental animals. Introduction of \textit{P. amarus} boosted the antioxidant defense capacity of these tissues in a dose-dependent manner. In addition, treatment with \textit{P. amarus} and standard chloroquine dose show schizontocidal activity in both early and established infectious conditions (Table 1). Onyesom et al.\textsuperscript{12} and Dapper et al.\textsuperscript{24} demonstrated the antiplasmodial activity of \textit{P. amarus}. Several other medicinal plants such as \textit{Pyrenacantha staudtii},\textsuperscript{20} \textit{Aspilia africana},\textsuperscript{30} \textit{Amaranthus spinosus} \textit{L.} and \textit{Andrographis paniculata} \textit{Burn.} \textit{f/Nees.},\textsuperscript{31} \textit{Piper betle}\textsuperscript{22} and \textit{Acacia auriculiformis} have also been shown to possess antimalarial activity. Chloroquine treatment did not augment the amounts of antioxidants as \textit{P. amarus} treatment and so could not protect the testes and ovaries against free radical challenge as evidenced by the MDA values (Tables 1 and 2) and Photomicrographs (Figures 1-18). Therefore, the identified compounds in the phytochemical profile of \textit{P. amarus}\textsuperscript{32} contain phytoconstituents with both antiplasmodial and antioxidant activities. Several researches have validated the antioxidant potential of \textit{P. amarus}. Kumar and Kuttan\textsuperscript{34} showed that \textit{P. amarus} methanolic extract increased antioxidant defense mechanism in mice and protected the animals from radiation-induced cellular damage. Arun and Balasubramanian,\textsuperscript{35} Syed et al.\textsuperscript{36} and Padmaja et al.\textsuperscript{37} showed the hepatoprotective value of \textit{P. amarus}. Reduction in testicular activities of the antioxidant enzymes as well as levels of GSH with a significant increase in the level of MDA as observed in this study is in line with the work of Nnodim and Nwanjo.\textsuperscript{38} The decrease in antioxidants could be associated with free radicals induced by the \textit{Plasmodium} parasites. Reactive oxygen species can interact with antioxidants and inactivate antioxidant enzymes producing a state of oxidative stress. The decrease in SOD and CAT activities could be attributed to oxidative inactivation of enzymes.\textsuperscript{39} The high MDA observed in the present study could bond with amino group of proteins to form intra- and inter-molecular crosslinks which could inactivate several membrane-bound enzymes. The increased levels of MDA in testes and ovaries of \textit{P. berghei} infected mice (Group 2, Tables 1 and 2) may indicate damage triggered by free radicals.\textsuperscript{39} Lipid peroxidation (LPO) is a degenerative mechanism of membrane component mediated through free radical production in the cell.\textsuperscript{41}
Table 1: Changes in ovarian antioxidants and malondialdehyde levels in *Plasmodium berghei* infected mice treated with *Phyllanthus amarus* and chloroquine.

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (units/mg protein)</th>
<th>CAT (μmole H₂O₂/min/mg protein)</th>
<th>GPx</th>
<th>GSH (mg/g tissue)</th>
<th>MDA (units/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.82 ± 0.37</td>
<td>12.72 ± 1.52</td>
<td>7.22 ± 0.82</td>
<td>12.32 ± 2.10</td>
<td>35.10 ± 3.70</td>
</tr>
<tr>
<td>2</td>
<td>1.38 ± 0.07</td>
<td>20.23 ± 2.84*</td>
<td>11.05 ± 1.34*</td>
<td>22.72 ± 2.04*</td>
<td>38.94 ± 3.51</td>
</tr>
<tr>
<td>3</td>
<td>1.30 ± 0.20</td>
<td>17.04 ± 1.70*</td>
<td>8.82 ± 0.19*</td>
<td>17.70 ± 1.20*</td>
<td>14.75 ± 2.13*</td>
</tr>
<tr>
<td>4</td>
<td>1.55 ± 0.08*</td>
<td>21.96 ± 2.94*</td>
<td>10.64 ± 1.51*</td>
<td>17.70 ± 3.63*</td>
<td>9.88 ± 0.52*</td>
</tr>
<tr>
<td>5</td>
<td>2.74 ± 0.72*</td>
<td>17.26 ± 1.66*</td>
<td>10.30 ± 1.36*</td>
<td>18.64 ± 1.50*</td>
<td>7.14 ± 1.34*</td>
</tr>
<tr>
<td>6</td>
<td>0.75 ± 0.47</td>
<td>12.26 ± 1.58</td>
<td>3.78 ± 0.83*</td>
<td>6.30 ± 2.30*</td>
<td>38.98 ± 6.33</td>
</tr>
<tr>
<td>7</td>
<td>1.42 ± 0.12</td>
<td>18.78 ± 1.22*</td>
<td>9.34 ± 0.46</td>
<td>16.32 ± 4.79</td>
<td>8.00 ± 1.60*</td>
</tr>
<tr>
<td>8</td>
<td>1.39 ± 0.10</td>
<td>20.30 ± 2.69*</td>
<td>9.46 ± 0.80*</td>
<td>18.04 ± 1.58*</td>
<td>9.40 ± 1.56*</td>
</tr>
<tr>
<td>9</td>
<td>1.98 ± 0.30*</td>
<td>21.70 ± 3.21*</td>
<td>14.20 ± 2.12*</td>
<td>19.32 ± 2.34*</td>
<td>4.18 ± 1.44*</td>
</tr>
</tbody>
</table>

SOD, Superoxide dismutase; CAT, Catalase; GPx, Glutathione peroxidase; GSH, Reduced glutathione, MDA, Malondialdehyde. *Significant at (p < 0.05) when compared with normal control values.

Table 2: Changes in levels of antioxidants and malondialdehyde in testes of mice infected with *Plasmodium berghei* and treated with *Phyllanthus amarus* and chloroquine.

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (units/mg protein)</th>
<th>CAT (μmole H₂O₂/min/mg protein)</th>
<th>GPx</th>
<th>GSH (mg/g tissue)</th>
<th>MDA (units/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.03 ± 0.16</td>
<td>12.66 ± 1.54</td>
<td>6.10 ± 0.84</td>
<td>12.62 ± 1.64</td>
<td>44.10 ± 1.31</td>
</tr>
<tr>
<td>2</td>
<td>0.07 ± 0.03</td>
<td>10.92 ± 0.96</td>
<td>2.26 ± 0.74</td>
<td>1.38 ± 0.24*</td>
<td>75.45 ± 0.07*</td>
</tr>
<tr>
<td>3</td>
<td>1.61 ± 0.41</td>
<td>13.10 ± 2.17</td>
<td>11.68 ± 0.59*</td>
<td>19.50 ± 3.51*</td>
<td>7.62 ± 1.73*</td>
</tr>
<tr>
<td>4</td>
<td>1.77 ± 0.50</td>
<td>18.38 ± 3.16</td>
<td>15.32 ± 2.59*</td>
<td>20.54 ± 2.71*</td>
<td>6.90 ± 1.97*</td>
</tr>
<tr>
<td>5</td>
<td>2.76 ± 0.73</td>
<td>27.93 ± 6.71*</td>
<td>25.14 ± 2.96*</td>
<td>19.40 ± 1.72*</td>
<td>4.76 ± 1.66*</td>
</tr>
<tr>
<td>6</td>
<td>6.30 ± 1.74*</td>
<td>42.13 ± 3.82*</td>
<td>41.97 ± 2.80*</td>
<td>34.40 ± 3.78*</td>
<td>3.32 ± 1.34*</td>
</tr>
<tr>
<td>7</td>
<td>5.66 ± 2.03*</td>
<td>38.14 ± 3.15*</td>
<td>53.40 ± 3.73*</td>
<td>42.74 ± 2.82*</td>
<td>4.24 ± 1.38*</td>
</tr>
<tr>
<td>8</td>
<td>5.16 ± 1.41*</td>
<td>35.34 ± 3.53*</td>
<td>63.84 ± 3.41*</td>
<td>37.30 ± 2.61*</td>
<td>2.38 ± 0.85*</td>
</tr>
<tr>
<td>9</td>
<td>8.20 ± 1.49*</td>
<td>44.10 ± 3.51*</td>
<td>64.17 ± 3.35*</td>
<td>55.42 ± 3.03*</td>
<td>1.62 ± 0.62*</td>
</tr>
</tbody>
</table>

SOD, Superoxide dismutase; CAT, Catalase; GPx, Glutathione peroxidase; GSH, Reduced glutathione, MDA, Malondialdehyde. *Significant at (p < 0.05) when compared with normal control values.

Table 3: Blood schizonticidal activity of *Phyllanthus amarus* in early and established *P. berghei* infection in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood schizonticidal activity of <em>Phyllanthus amarus</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early infection</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>52.0 ± 4.0</td>
</tr>
<tr>
<td>4</td>
<td>58.0 ± 6.0</td>
</tr>
<tr>
<td>5</td>
<td>64.0 ± 5.0</td>
</tr>
<tr>
<td>6</td>
<td>68.0 ± 7.0</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
</tr>
</tbody>
</table>

SOD, Superoxide dismutase; CAT, Catalase; GPx, Glutathione peroxidase; GSH, Reduced glutathione, MDA, Malondialdehyde. *Significant at (p<0.05) when compared with normal control values.
Figure 1: Histology of ovary of the control mice (Group 1). Features and ovarian cell appear normal. Microscopic magnification = x100, (H and E staining).

Figure 2: Histology of ovary cells infected with *P. berghei* (Group 2). Pathological feature show distorted linings of the zona pellucida, the primary follicle and the zona granulosa. Microscopic magnification = x100, (H and E staining).

Figure 3: Histology of ovarian cells infected with *P. berghei* and treated with 100 mg/kg *Phyllanthus amarus* for 7 days (Group 3). Features show a mild improvement in the distorted lining of the zona pellucida and zona granulosa. Microscopic magnification = x100, (H and E staining).

Figure 4: Histology of ovarian cells infected with *P. berghei* and treated with 200 mg/kg *Phyllanthus amarus* for 7 days (Group 4). Features indicate moderate improvement in the lining of the zona pellucida and zona granulosa, an evidence of repair and recovery. Microscopic magnification = x100, (H and E staining).

Figure 5: Histology of ovarian cells infected with *P. berghei* and treated with 300 mg/kg *Phyllanthus amarus* for 7 days (Group 5). The zona pellucida and zona granulosa appear perfectly normal. This dose of *P. amarus* protects ovarian cells from oxidative assault by *P. berghei*. Microscopic magnification = x100, (H and E staining).

Figure 6: Histology of ovarian cells infected with *P. berghei* and treated with 5 mg/kg chloroquine for 7 days (Group 6). Features show moderate distortion in the linings of the zona pellucida and zona granulosa. Microscopic magnification = x100, (H and E staining).
Figure 7: Histology of ovarian cells not infected but treated with 100 mg/kg of *Phyllanthus amarus* (Group 7). Features appear normal when compared with the control. Microscopic magnification = x100, (H and E staining).

Figure 8: Histology of ovarian cells not infected but treated with 200 mg/kg of *Phyllanthus amarus* (Group 8). Features show cells replenishment. Microscopic magnification = x100, (H and E staining).

Figure 9: Histology of ovarian cells not infected but treated with 500 mg/kg of *Phyllanthus amarus* (Group 9). Cell features appear normal and invigorated when compared with the control. Microscopic magnification = x100, (H and E staining).

Figure 10: Histology of testes for control mice (Group 1). Features indicate normal linings of the spermatogonia, spermatocytes, leydig cells and the basement membrane. Microscopic magnification = x100, (H and E staining).

Figure 11: Histology of testes for mice infected with *P. berghei* (Group 2). Features show total distortion in the lining of the basement membrane, the spermatogonia, spermatocytes and leydig cells. Microscopic magnification = x100, (H and E staining).

Figure 12: Histology of testes for mice infected with *P. berghei* (Group 3) and treated with 100 mg/kg of *Phyllanthus amarus* for 7 days. Features indicate mild changes in the tissue. Microscopic magnification = x100, (H and E staining).
Figure 13: Histology of testes for mice infected with *P. berghei* (Group 4) and treated with 200 mg/kg of *Phyllanthus amarus* for 7 days. Features indicate significant improvement in the tissue distortions when compared with *P. berghei* infection only. Microscopic magnification = x100, (H and E staining).

Figure 14: Histology of testes for mice infected with *P. berghei* (Group 5) and treated with 300 mg/kg of *Phyllanthus amarus* for 7 days. Features of the cell appeared normal when compared with the control group. Microscopic magnification = x100, (H and E staining).

Figure 15: Histology of testes for mice infected with *P. berghei* (Group 6) and treated with chloroquine for 7 days. Features show moderate distortion in the cells of the testes when compared with the control. Microscopic magnification = x100, (H and E staining).

Figure 16: Histology of testes for mice without *P. berghei* infection (Group 7) but administered with 100 mg/kg *Phyllanthus amarus* for 7 days. No damage was observed in the tissue. Microscopic magnification = x100, (H and E staining).

Figure 17: Histology of testes for mice without *P. berghei* infection (Group 7) but administered with 200 mg/kg *Phyllanthus amarus* for 7 days. Cell features appear normal. Microscopic magnification = x100, (H and E staining).

Figure 18: Histology of testes for mice without *P. berghei* infection (Group 7) but administered with 300 mg/kg *Phyllanthus amarus* for 7 days. Cell were protected and replenished. Microscopic magnification = x100, (H and E staining).
Increased LPO implies membrane instability which correlates with altered tissue membrane as observed in the present study. In the ovarian cells, infection with P. berghei, results in the generation of highly reactive oxygen species (ROS). However, P. amarus was shown to be able to greatly nullify this effect. It was able to increase the activity of the antioxidant enzymes necessary for the reduction of the already produced ROS. This is in line with the study of Kumar and Kuttan who reported increases in the activities of SOD, CAT, GST, GPx, glutathione reductase (GR) in both blood and tissue which were initially reduced by radiation treatment. 
P. amarus has been shown to possess several active ingredients. A variety of tannins purified from P. amarus was found to be potent inhibitors of protein kinases. The presence of several lignans such as phyllanthin and hypophyllantin, polyphenols, flavonoids and some ellagitanins isolated from P. amarus could also be responsible for the antioxidant activity exhibited by the extract. Many plant phenolic compounds show excellent antioxidant activities and are good inhibitors of lipid peroxidation. Although the exact mechanism of P. amarus action needs to be elucidated, the combined action of these components could be responsible for its antioxidant and antiplasmodial properties.

**Conclusion**

The current study has shown the antioxidant and antiplasmodial potential of P. amarus. P. amarus exerts its antioxidant effect by countering the formation or reactivities of free radicals induced by P. berghei infection via participation of the plant's phytochemicals. This offered a measure of protection to the ovaries and testes of plasmodium parasites infected mice. However, the mechanism of P. amarus antimalarial potential involving its chemical compounds is still emerging.

**Conflict of interest**

The authors declare no conflict of interest.

**Authors' Declaration**

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

**References**


