Suppressive and Prophylaxis Activities of Ethanol Leaf Extract of *Musa paradisiaca* on Liver Antioxidant Profile of *Plasmodium berghei* Infected Mice

Ibitoroko Maureen George-Opuda¹*, Adebayo Olugbenga Adegoke, Othuke Bensandy Odeghe, Abimbola Temitayo Awopeju, Kemzi Nosike Elechi-Amadi, and Olugbenga Emmanuel Bamigbowu

ABSTRACT

This study evaluated the in vivo Prophylactic and Suppressive antimalarial activities of a locally formulated herbal antimalarial therapy, dry plantain leaf extract (*Musa paradisiaca*) on liver antioxidant profile of mice infected with *Plasmodium berghei*. Prophylactic assessment involved six groups (control, *P. berghei* infected, *P. berghei* infected and artesunate treated, *P. berghei* infected and oral treatment with 250, 500 and 1000 mg/kg *Musa paradisiaca* leaf extract) using Prophylactic model. Another six groups following the same order was used for suppressive assessment using suppressive model. The rats were sacrificed on the 10th day, and blood samples collected through cardiac puncture for Catalase, Glutathione Peroxidase, Glutathione and Malondialdehyde. Blood smears was evaluated microscopically for parasitaemia. Data was analyzed using SPSS version 21. Catalase (umol/ml/mins) showed considerable variation in the control, *P. berghei* infected, and treatment groups; 24.62 ± 0.99, 10.04 ± 0.50, 23.97 ± 0.00 suppressive, and prophylaxis assessments respectively. The Glutathione Peroxidase (u/l) also showed significant decrease in the *P. berghei* infected group (205.22 ± 4.61) when compared with control 332.34 ± 0.64, and treatment groups 317.34 ± 0.00, 319.46 ± 0.64 and 317.76 ± 0.15 respectively (p < 0.05). Malondialdehye in the *P. berghei* infected group was increased 61.65 ± 1.72 when compared with the control and other treatment groups (p < 0.05). Protein (g/dl) decreased in the *P. berghei* infected group (10.22 ± 0.00) when compared to control and treatment groups (p < 0.05). The study showed that *P. berghei* elevated liver oxidation parameters while *Musa paradisiaca* leaf increased some antioxidants parameters, suggesting prophylaxis and suppressive properties.

Keywords: Antioxidant, Liver, *Musa paradisiaca*, *Plasmodium berghei*.

1. Introduction

Malaria infection is still a serious health issue especially in Africa. This is more so when certain factors such as the number of people affected, the morbidity, and mortality level associated with the malaria (World Health Organization, 2016). The rodent parasite *Plasmodium berghei* is one of the well-employed models in malaria related research, and this includes analysis on severe pathology associated with malaria infections. It has been reported that *Plasmodium berghei* can induce a number of disease states in rodents (Blandine et al., 2010). Given this serious challenge, and the fact that there have been reports of resistance to orthodox antimalarials, attention is being shifted to research into the antimalarial potentials of herbs. This is expected to lead to the development of antimalarial substances from the herbal sources (Wichmann et al., 2004). Furthermore, there are claims that these plant sources have immense curative potential, including the treatment of malaria infection, especially in developing countries.
is therefore important that such claims, which has led to the increased use of these plants and plant parts, be subjected to scientific investigation in order to scientifically validate these claims (Adebayo & Krettli, 2011).

Plants are a rich source of bioactive compounds that reportedly have beneficial potentials, including antimalarial potentials (Newman et al., 2003).

*Musa paradisiaca* is a monoherbaceous plant that is found in most tropical and subtropical regions. The plant has a lot of medicinal values, as its parts have been used in traditional medical practices in the treatment of several health condition, including diabetes mellitus, ulcer, and other conditions. This is because the plant and its parts possess antioxidant, anti-inflammatory, antibacterial, antifungal, and even hepatoprotective properties (Lavanye et al., 2016). Some authors have reported that the extracts of *Musa paradisiaca* exhibited anti-inflammatory and anti-oxidant potentials in experimental rats (Vijayakumar et al., 2008; Shodehinde & Oboh, 2013).

This study focused on evaluating the antimalarial potential of the extract of *Musa paradisiaca* leaf, as well as and the biochemical effect of *Musa paradisiaca* on selected liver antioxidants using albino mice.

### 2. Materials and Methods

#### 2.1. Animal

A total of 120 healthy albino mice of about 2–3 months old weighing between 13–36 g obtained from animal house of the Federal University of Technology, Owerri, Nigeria were used for the study. They were kept in the animal house of Madonna University, Elele campus and were housed in well-ventilated aluminum cages and placed under room temperature and 12-hour light/darkness cycles. The mice had free access to clean drinking water and feed (pelleted starter feed produced by Vital Feeds). They were allowed for two weeks to acclimatize before administration. They were maintained in accordance with their commendations of the guide for the care and use of laboratory animals and experimental protocol was approved by the institution.

#### 2.2. Extraction and Preparation of Plant Materials

The fresh leaves of *Musa paradisiaca* were obtained from a plantain plantation in Elele, Rivers State, Nigeria. They were cut into pieces, washed, and dried under shade. The dried pieces were grounded into fine powder using a manual grinder; 10 kg of the grounded powder was soaked in 10 litres of 80% ethanol for 72 hours with intermittent stirring of the solution. The mixture was subsequently filtered through Whatman filter paper (125 mm). The extract was concentrated using a rotary evaporator at 45 °C and then dried with a water bath at 39 °C to yield 40 g of dark semisolid extract and kept at 0–4 °C until needed for use.

#### 2.3. Parasite Inoculation

A chloroquine-sensitive strain of *Plasmodium berghei* (NK 65 strain) parasite used in this study was obtained from the Department of Pharmacology and Toxicology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Rivers State, Nigeria. The *Plasmodium berghei* was sustained by constant re-infestation of parasitized erythrocytes which were sourced from a donor-infected mouse by the tail via a heparinized syringe and made up to 20 ml with normal saline. The animals were inoculated with 0.2 ml of infected blood suspension. The donor mice were monitored for signs of infection which include; lethargy, anorexia, shivering and heat-seeking environment. Parasitemia was monitored daily by microscopic examination of Giesma stained thick film and viewed at ×100 objective. Furthermore, malaria parasite infection was detected by thin film stained with Leishman stain by parasite count using the formula:

\[
\text{Parasite density} = \frac{\text{Number of parasites counted} \times 100}{\text{white blood cells/Number of white blood cells counted}}
\]

#### 2.4. Acute Toxicity Study (LD₅₀)

The toxicity of the crude extract of *Musa paradisiaca* leaf was evaluated using mice that were not infected with *P. berghei*. The mice were two months old and weighed 18–20 g. The toxicity study was done using the Locke’s method as modified (Locke, 1983).

#### 2.5. Evaluation of Prophylactic Activity (Repository Test)

Prophylactic activity of *M. paradisiaca* was determined using the method described by Peters (1967). In this method, sixty mice randomly divided into six groups of 10 mice was assigned to control group, artesunate treated, *P. berghei* infected and the experimental groups of 250,500 and 1000 mg/kg/day body weight *Musa paradisiaca* leaf extract. The control was administered diet and water ad libitum, the artesunate treated was given 50 mg artesunate per kg body weight intraperitoneally while the experimental groups were administered with 250, 500 and 1000 mg/kg/day body weight *Musa paradisiaca* leaf extract and the last group was infected with *P. berghei*. Treatments were initiated on day 0 and continued until day 4; the mice were all infected with the parasite. Blood smears were then made from each mouse 72 hours after treatment (Abatan & Makinde, 1986) while the increase or decrease in parasitaemia was then determined.

#### 2.6. Suppressive Test (Schizontocidal Activity on Early Infection)

This was done to determine the schizontocidal activity of both the extract and artesunate (orthodox drug) against early infection of *P. berghei* using the method of Knight and Peters (1980).

Briefly, the mice were inoculated intraperitoneally, with 0.2 ml of infected blood containing 1 × 10⁷ *P. berghei*—parasitized erythrocytes, on the first day (designated Day 0) except the control. The animals were divided into six groups of ten mice each and orally administered shortly after inoculation with 250, 500 and 1000 mg/kg/day doses of the *Musa paradisiaca* leaf extract, artesunate 50 mg/kg/day and a 250 ml volume of distilled water to the control for five consecutive days, (day 0 to day 4) while the last group was only given food and water after administration of *P. berghei*. On the 6th day (designated Day 5), the
tails of the mice were pierced in order to obtain drops of blood used for making thin films. These thin blood films were then fixed with methanol, and then stained with 10% Giemsa stain at pH 7.2 for 10 minutes. The films were allowed to dry then vied microscopically. The malaria parasitaemia was determined by counting the number of parasitized red cells out of 200 red cells in random fields of the microscope.

The chemosuppression was calculated using the formula: 100 [(N−P)/N], where N is the average percentage parasitaemia in the negative control group and P is the average parasitaemia in the test/standard group.

2.7. Biochemical Analysis

Catalase estimation was done according to method of Mahmoud and Haider (2018) as modified by Sigma Aldrich diagnostic using Colorimetric method. This assay method is based on the principle of the measurement of the hydrogen peroxide substrate remaining after the action of catalase. First, the catalase converts hydrogen peroxide to water and oxygen (catalytic pathway) and then this enzymatic reaction is stopped with sodium azide. An aliquot of the reaction mix is then assayed for the amount of hydrogen peroxide remaining by a colorimetric method. The colorimetric method uses a substituted phenol (3,5-dichloro-2-hydroxybenzenesulfonic acid), which couples oxidatively to 4-aminoantipyrine in the presence of hydrogen peroxide and horseradish peroxidase (HRP) to give red quinoneimine dye (N-(4-antipyril)-3-chloro-5-sulfonate- benzoquinone monoimine) that absorbs at 520 nm.

The assay reaction was performed at room temperature (25 °C). The Assay Buffer, Colorimetric Assay Substrate Solution, and Color Reagent were allowed to equilibrate to room temperature. Into a test tube, 25 μl of sample and 75 μl of assay buffer were dispensed, then the reaction was started by addition of 25 μl of substrate B to the test tube. It was mixed by inversion and incubated at 25 °C for 15 minutes. 825 μl of the stop solution was added and mixed. 10 μl aliquot of the mixture was removed and added to another test tube and 1 ml of chromogen reagent was added and mixed well. Then it was allowed for 15 minutes at room temperature for color development. Change in absorbance was read at 520 nm wavelength.

Glutathione peroxidase (GPX) estimation was carried out according to method of Charmagnol et al. (1983) as modified by Sigma Aldrich diagnostic using Spectrophotometric method. The principle showed that Glutathione peroxidase catalysts the oxidation of Glutathione (GSH) by cumene hydroperoxides. The oxidized glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH. In this reaction the NADPH is oxidized to NADP+ simultaneously. The decrease in absorbance at 340nm is then measured.

Into a clean microcuvette 20 μl of sample was added and 20 μl of distilled water into another cuvette (Reagent Blank), then 1 ml of working reagent was added to each cuvette. 40 μl of cumene hydroperoxide solution was added to each cuvette. It was mixed and initial absorbances of the sample and reagent blank were read after 1 minute and timer was started simultaneously. It was read again after 1 and 2 minutes. The reagent blank value was subtracted from that of the sample.

Protein estimation was done using Biuret method as outlined by Henry (1974) and modified by Fortress diagnostic was used. Copper ions react in alkaline solution, with protein peptide bonds to give a purple colored biuret complex. The amount of complex formed is directly proportional to the amount of protein in the specimen.

Into three test tubes labelled test, standard and blank, 1000 μl of biuret reagent was added to all. A 20 μl sample, standard, and distilled water was added to a respective test tube. The tubes were mixed thoroughly and incubated for 10 minutes at room temperature. Their absorbance was read spectrophotometrically at 546 nm wavelength. The unknown was extrapolated by comparing the absorbance of unknown with absorbance and concentration of standard.

Malondialdehyde (MDA) estimation was done by Colorimetric method. The principle of this assay is based on the reaction of a chromogenic reagent, 2-thiobarbituric acid, with MDA at 25 °C. One molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid via a Knoevenagel-type condensation to yield a chromophore with absorbance maximum at 532 nm.

The Free MDA and Total MDA were estimated as shown below.

2.7.1. Free MDA

Into glass tubes labelled standards, samples and blank, 200 μl of standard, sample and 200 μl of indicator solution were added. This was followed by addition of 200 μl of indicator solution were added respectively and mixed well.

After the standards, samples and blanks were mixed; it was allowed to react for 45 minutes at room temperature. Then 300 μl was transferred to a microplate and the absorbance of the resulting solution was measured at 532 nm. The pink color is stable for several hours at room temperature.

2.7.2. Total MDA

Into glass tubes labelled standards, samples and blank, 200 μl of standard, sample and 200 μl of indicator solution were added. This was followed by addition of 200 μl of indicator solution were added respectively and mixed well. Sample was heated at 65 °C for 45 minutes, Then 300 μl was transferred to a microplate and the absorbance of the resulting solution was measured at 532 nm.

Reduced glutathione (GSH) estimation was done by Spectrophotometric method using Ellman reagent (Ellman, 1959) as modified by Sigma Aldrich diagnostic. The principle of the method showed that 5,5′-dithiobis-(2-nitrobenzoic acid) is reduced by SH groups to form 1 mole of 2-nitro-5-mercaptopbenzoic acid per mole of SH. The nitromercaptobenzoic acid anion has an intense yellow color which when measured at a wavelength of 412 nm can be used to measure SH groups.

Into a microcuvette, 100 μl of standard and samples were dispensed respectively. Then 880 μl of GSH dilution buffer was added to the microcuvette and 20 μl of GSH chromogen was added to the microcuvette respectively, it was mixed well and absorbance of resulting solution was measured at 412 nm wavelength within 5 minutes.
TABLE I: SUPPRESSIVE ACTIVITY OF ETHANOL LEAF EXTRACT OF MUSA PARADISIACA ON LIVER ANTIOXIDANT PROFILES OF PLASMODIUM BERGHEI INFECTED MICE.

<table>
<thead>
<tr>
<th>Group</th>
<th>Catalase (umol/ml/mins)</th>
<th>Glutathione peroxidase(u/l)</th>
<th>Glutathione (mM)</th>
<th>Malondialdehyde (uM)</th>
<th>Protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.62 ± 0.99</td>
<td>332.34 ± 0.64</td>
<td>1.60 ± 0.12</td>
<td>16.93 ± 3.59</td>
<td>22.37 ± 1.87</td>
</tr>
<tr>
<td>P. berghei treated</td>
<td>10.04 ± 0.50</td>
<td>205.22 ± 4.61</td>
<td>0.64 ± 0.09</td>
<td>61.65 ± 1.72</td>
<td>7.91 ± 0.13</td>
</tr>
<tr>
<td>Artesunate</td>
<td>22.11 ± 0.87</td>
<td>270.66 ± 7.22</td>
<td>1.50 ± 0.23</td>
<td>28.29 ± 0.91</td>
<td>13.13 ± 1.35</td>
</tr>
<tr>
<td>250 mg extract</td>
<td>21.57 ± 0.00</td>
<td>317.34 ± 0.00</td>
<td>1.33 ± 0.00</td>
<td>31.14 ± 0.00</td>
<td>10.22 ± 0.00</td>
</tr>
<tr>
<td>500 mg extract</td>
<td>20.15 ± 0.13</td>
<td>319.46 ± 0.64</td>
<td>1.33 ± 0.49</td>
<td>25.17 ± 0.28</td>
<td>13.14 ± 1.95</td>
</tr>
<tr>
<td>1000 mg extract</td>
<td>22.55 ± 0.43</td>
<td>315.76 ± 0.15</td>
<td>1.42 ± 0.02</td>
<td>26.16 ± 0.05</td>
<td>11.03 ± 0.04</td>
</tr>
</tbody>
</table>

Note: * = Compare with the control, ** = Compare with the P. berghei treated.

TABLE II: PROPHYLAXIS ACTIVITY OF ETHANOL LEAF EXTRACT OF MUSA PARADISIACA ON LIVER ANTIOXIDANT PROFILES OF PLASMODIUM BERGHEI INFECTED MICE.

<table>
<thead>
<tr>
<th>Group</th>
<th>Catalase (umol/ml/mins)</th>
<th>Glutathione peroxidase(u/l)</th>
<th>Glutathione (mM)</th>
<th>Malondialdehyde (uM)</th>
<th>Protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.62 ± 0.99</td>
<td>332.34 ± 0.64</td>
<td>1.60 ± 0.12</td>
<td>16.93 ± 3.59</td>
<td>22.37 ± 1.87</td>
</tr>
<tr>
<td>P. berghei treated</td>
<td>10.04 ± 0.50</td>
<td>205.22 ± 4.61</td>
<td>0.64 ± 0.09</td>
<td>61.65 ± 1.72</td>
<td>7.91 ± 0.13</td>
</tr>
<tr>
<td>Artesunate</td>
<td>22.11 ± 0.87</td>
<td>270.66 ± 7.22</td>
<td>1.50 ± 0.23</td>
<td>28.29 ± 0.91</td>
<td>13.13 ± 1.35</td>
</tr>
<tr>
<td>250 mg extract</td>
<td>23.41 ± 0.00</td>
<td>301.59 ± 0.00</td>
<td>1.44 ± 0.00</td>
<td>33.29 ± 0.00</td>
<td>19.02 ± 0.00</td>
</tr>
<tr>
<td>500 mg extract</td>
<td>22.19 ± 0.19</td>
<td>305.66 ± 1.36</td>
<td>1.33 ± 0.44</td>
<td>25.17 ± 0.28</td>
<td>13.14 ± 1.95</td>
</tr>
<tr>
<td>1000 mg extract</td>
<td>23.97 ± 0.00</td>
<td>309.45 ± 0.00</td>
<td>1.24 ± 0.00</td>
<td>23.29 ± 0.00</td>
<td>11.07 ± 0.04</td>
</tr>
</tbody>
</table>

Note: * = Compare with the control, ** = Compare with the P. berghei treated.

3. Statistical Analysis

Data obtained were subjected to statistical analysis using statistical package for social science version 21 using statistical tools such as t-test and analysis of variance (ANOVA). Results were expressed as Mean ± Standard Deviation (X ± SD). The values of P < 0.05 was considered significant.

4. Result

The P. berghei reduced the Catalase (µmol/ml/mins) of 24.62 ± 0.99 in control to 10.04 ± 0.50. Treatment with 250 mg extract, 500 mg extract and 1000 mg extract increased the Catalase (µmol/ml/mins) to 21.57 ± 0.00, 20.15 ± 0.13 and 22.55 ± 0.43, respectively.

The Glutathione Peroxidase (uM/l) was 332.34 ± 0.64 in control, 205.22 ± 4.61 in P. berghei. Administration of 250 mg extract, 500 mg extract and 1000 mg extract increased the Glutathione Peroxidase (µ/l) to 317.34 ± 0.00, 319.46 ± 0.64 and 315.76 ± 0.15, respectively. The Glutathione (mM) was 1.60 ± 0.12 in control, 0.64 ± 0.09 in P. berghei treated while administration of 250 mg extract, 500 mg extract and 1000 mg extract increased the Glutathione (Mm) to 1.33 ± 0.00, 1.35 ± 0.49 and 1.42 ± 0.02, respectively. The Malondialdehyde (µM) was 16.93 ± 3.59 in control, 61.65 ± 1.72 in P. berghei. Administration of 250 mg extract, 500 mg extract and 1000 mg extract increased the Malondialdehyde to 31.14 ± 0.00, 25.17 ± 0.28 and 26.16 ± 0.05, respectively. P. berghei reduced Protein (g/dl) concentration of 22.37 ± 1.87 in control to 7.91 ± 0.13 while administration of 250 mg extract, 500 mg extract and 1000mg extract increased the protein to 10.22 ± 0.00, 10.14 ± 0.19 and 11.03 ± 0.04, respectively as shown below in Table I.

The P.berghei reduced the Catalase (µmol/ml/mins) of 24.62 ± 0.99 in control to 10.04 ± 0.50. Treatment with 250 mg extract, 500 mg extract, and 1000 mg extract increased the Catalase (µmol/ml/mins) to 23.41 ± 0.00, 22.19 ± 0.19 and 23.97 ± 0.00, respectively. The Glutathione Peroxidase (µ/l) was 332.34 ± 0.64 in control, 205.22 ± 4.61 in P. berghei. Administration of 250 mg extract, 500 mg extract, and 1000 mg extract increased the Glutathione Peroxidase (µ/l) to 301.59 ± 0.00, 305.66 ± 1.36 and 309.45 ± 0.00, respectively. The Glutathione (mM) was 1.60 ± 0.12 in control, 0.64 ± 0.09 in P. berghei treated while administration of 250 mg extract, 500 mg extract and 1000 mg extract increased the Glutathione (mM) to 1.44 ± 0.00, 1.33 ± 0.44 and 1.24 ± 0.00, respectively. The Malondialdehyde (µM) was 16.93 ± 3.59 in control, 61.65 ± 1.72 in P. berghei. Administration of 250 mg extract, 500 mg extract, and 1000 mg extract decreased the Malondialdehyde to 33.29 ± 0.00, 25.17 ± 0.28 and 23.29 ± 0.00, respectively. P. berghei reduced protein (g/dl) concentration of 22.37 ± 1.87 in control to 7.91 ± 0.13 while administration of 250 mg extract, 500 mg extract, and 1000 mg extract increased the protein to 19.02 ± 0.00, 10.14 ± 0.09 and 11.07 ± 0.00, respectively as shown below in Table II.

5. Discussion

Malaria is an important health issue in most African countries due to the rate of morbidity and mortality it caused in most countries (World Health Organization, 2016). P. berghei is similar to malaria parasites of mammals, including the four human malaria parasites is transmitted by Anopheles mosquitoes and infects the liver after being injected into the bloodstream by a bite of an infected female mosquito. After a few days of development and multiplication the parasites invade erythrocytes also called red blood cells leading to pathology such as anaemia and damage of essential organs of the host such as lungs.
liver, spleen (Franks-Fayard, 2010) therefore it is used in this study.

Plants are a rich source of bioactive compounds that reportedly have beneficial potentials, including antimalarial potentials (Adebayo & Krettli, 2011). Some authors have reported that the extracts of *Musa paradisiaca* exhibited anti-inflammatory and anti-oxidant potentials in experimental rats (Vijayakumar et al., 2008; Shodehinde & Oboh, 2013) hence it is used in this study as an antimalarial and antioxidant.

The result of the study showed that *P. berghei* caused reduction of Catalase, Glutathione Peroxidase Gluthathione with increase Malondialdehyde. This is suggestive that *P. berghei* caused Liver oxidation by increasing Malondialdehyde an indicator of lipid peroxidation.

The result of the study showed that suppressive extract of *Musa paradisiaca* increased Catalase, Glutathione Peroxidase Gluthathione with a decrease Malondialdehyde. This may be due to the antioxidant potential of the plantain leaf extracts. Plantain leaves have been reported to contain high amounts of phytochemicals with various pharmacological activities as well as minerals and amino acids (Turgumbayeva et al., 2022).

The result of the study further showed dose dependent increase in Catalase, Glutathione Peroxidase Gluthathione with decrease Malondialdehyde concentration with Prophylaxis extract of *Musa paradisiaca*. This is suggestive that Prophylaxis extract reduced the liver oxidation caused by *P. berghei*. The results from this study could be attributed to the presence of these phytochemical constituents of the plantain leaf extract. Herbal substances are known to exhibit inhibitory effects on the production of inflammatory markers (Mahdi et al., 2018) and also exhibit anti-inflammatory effects (Turgumbayeva et al., 2022). Thus, the increases in the antioxidant parameters could be due to the antioxidant and anti-inflammatory potentials of the extract. These findings agree with a similar work by Okorie et al. (2023) using *Moringa oleifera* Lam seed extract.

6. Conclusion

The result of the study has shown that *P. berghei* caused Liver oxidation. Treatment with Prophylaxis and suppressive extract of *Musa paradisiaca* reversed the liver oxidation.

Acknowledgment

The authors appreciate the staff of animal house of Madonna University, Elele Campus, Nigeria.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

References


