Antimalarial Activity and Effect of Ethanol Extract of *Pleiocarpa mutica* Leaves on Some Haematological Indices of *Plasmodium berghei*-Infected Mice

O.C. Enechi, B.C. Okenu and Uroko Robert Ikechukwu

**Abstract:** The present study evaluated antimalarial activity and the effect of ethanol extract of *Pleiocarpa mutica* leaves on some haematological indices of *Plasmodium berghei*-infected mice. A total of thirty mice (both infected (test) and non-infected (control)) consisting of six groups (of five mice each) were used in the study. Group 1 served as the negative control (non-parasitized), group 2 as the positive control (parasitized but not treated) and group 3, standard control (malaria-infected mice treated with artesunate). Groups 4, 5 and 6 were malaria-infected mice treated with 200, 300 and 400 mg/kg body weight of the ethanol extract of *Pleiocarpa mutica* leaves respectively. The acute toxicity study of the extract showed that it is relatively safe with LD₅₀ above 5000 mg/kg body weight. The phytochemical analysis of the *Pleiocarpa mutica* leaves revealed its richness in bioactive components, most especially flavonoids, alkaloids and terpenoids which have been shown to possess antimalarial properties. No parasitaemia was observed in the negative control while it was observed that there was a daily increase in percentage parasitaemia in the positive control group. However, there was a significant (p < 0.05), dose-dependent reduction in percentage parasitemia in the parasitized-treated groups when compared with the positive control as observed in day 2 and day 3 post-treatment. The decreases in percentage parasitaemia observed in the extract-treated groups were comparable to that of group 3 (treated with standard antimalarial drug). The haematological analysis revealed that infection induced significant (p<0.05) decreases in packed cell volume (PCV), haemoglobin concentration (Hb), the white blood cell count (WBC) and red blood cell count (RBC) of the positive control group when compared to parasitized-treated groups. The findings suggest that *P. mutica* leaves have anti-malarial activity and ameliorative effects on altered haematological indices in malaria parasite-infected mice.

**Key words:** *Pleiocarpa mutica* (**P. mutica**) Leaves - *Plasmodium berghei* - Antimalarial Activity - Haematological Indices - Ethanol Extract

**INTRODUCTION**

In Africa, malaria stands as the most common tropical disease confronting her inhabitants and a significant number of deaths due to malaria infection [1-3]. *Plasmodium berghei*, a rodent version of human malaria parasite (*Plasmodium falciparum*), has been used in the development of an experimental malaria model [4]. Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathogenesis [5]. In Nigeria and sub-Saharan Africa, one in five children die before they are five and 75% of those deaths are attributed to malaria [6]. Resistance of *Plasmodium falciparum* to commonly used antimalarial drugs is increasing in Nigeria as in other parts of Africa [7,8]. Pure products have been isolated from plants amongst which are those with antimalarial activities comparable to that of chloroquine on sensitive and resistant strains of *Plasmodium falciparum* [9]. Hence, the need to source antimalarial drugs from plants such as *Pleiocarpa mutica*.

*Pleiocarpa mutica* is an evergreen shrub widely distributed in tropical Africa. In southeast Nigeria, the plant extract is used in folk medicine for the treatment...
of malaria and many other ailments. In Sierra Leone, ground bark is rubbed on the body against fever. In Côte d’Ivoire, the decoction of the grated bark is used to treat stomach pains as well as oedema of the legs. Also, decoction of roots, leaves and stem are used for hemorrhoids, malaria, kidney disease and liver infections. In Ghana, the roots are taken in decoction as a febrifuge, antimalarial and to treat jaundice and convulsions [10].

Previous studies demonstrated the antiplasmodial [11] and hepatoprotective [12] activities of alkaloids isolated from the roots of Pleiocarpa mutica. The present study is a scientific approach to re-establish the traditional uses of the plant as antimalarial drug and to evaluate the effect of the plant leaf extract on some haematological indices in plasmodium berghei-infected mice.

**MATERIALS AND METHODS**

**Plant Materials:** Fresh leaves of Pleiocarpa mutica were harvested from Enugu-Ezike town in Igboeze-North, Enugu State, Nigeria and were identified by Mr. Alfred Ozioko of the Bioresources Development and Conservation Programme Centre, Nsukka, Enugu State, Nigeria.

**Animals:** Forty eight male albino mice (weighing 18-30g) were obtained from the Animal House, Department of Zoology, Faculty of Biological Sciences, University of Nigeria, Nsukka and were acclimatized for 14 days under standard environmental conditions with a 12 hour light/dark cycle and were maintained on standard feed (vital feed) and water ad libitum. The laboratory animals were used in accordance with laboratory practice regulation and the principle of humane laboratory animal care as documented by Zimmermann [13].

**Methods**

**Extraction of the Plant Material:** The leaves of Pleiocarpa mutica were washed with clean water to remove dirt and sand, drained and chopped. It was dried under shade for 3 weeks and then pulverized into fine powder. A quantity of 500g of the powdered stem bark was macerated in 1.5 liters of absolute methanol for 24 hours. The extract was filtered out through Whatman no. 1 filter paper and the filtrate (extract) was concentrated under reduced pressure using a rotary evaporator and subsequently evaporated to dryness on water bath at 60°C to brown dry residue and stored in refrigerator at 4°C until used.

**Experimental Design**

**Acute and Sub-Acute Toxicity:**

After the 14 days acclimatization, eighteen mice were used for acute toxicity study and thirty mice divided into six (6) groups of five (5) mice each were used for the antimalarial and haematological studies. The groupings include the followings:

- **Group 1:** Negative control- Not parasitized (Not treated).
- **Group 2:** Positive control - Parasitized mice (Not treated).
- **Group 3:** Standard control- Parasitized mice + Artesunate.
- **Group 4:** Parasitized mice + Pleiocarpa mutica leaf extract (200 mg/kg. b. wt)
- **Group 5:** Parasitized mice + Pleiocarpa mutica leaf extract (300 mg/kg. b. wt)
- **Group 6:** Parasitized mice + Pleiocarpa. mutica leaf extract (400 mg/kg. b. wt)

Parasitaemia counts were determined every 24 h following treatment with the standard drug (Artesunate) and crude ethanol extract of Pleiocarpa mutica leaves to respective groups for 3 days duration. Blood samples of the mice were collected by ocular puncture using heparinized capillary tubes for haematological study.

**Preparation of Malaria Parasite:** Standard innoculum of Plasmodium berghei was prepared from donor mouse with Plasmodium berghei-parasitized erythrocytes and parasitaemia was established through microscopic examination of thin blood film from Plasmodium berghei donor mouse under oil immersion at x100 magnification and measured as percentage erythrocytes. Each animal was infected with a standard innoculum of 10⁸ parasitized erythrocytes suspension in phosphate buffered saline (0.2ml) from donor mouse.

**Phytochemical Analysis:** The methods of Trease and Evans and Harborne [14,15], were used for both qualitative and quantitative phytochemical analyses.

**Determination of Acute Toxicity (LD₅₀) of the Ethanol Extract:** The acute toxicity of the ethanol extract of P. mutica leaves was determined according to Lorke [16].

**Determination of Percentage Malaria Parasitaemia:**
The percentage malaria parasitaemia (Mp+) was determined according to the method described by Dacie and Lewis [17].
Determination of Haematological Parameters:
The haematological parameters: packed cell volume, haemoglobin concentration, platelet count, red blood cell count, white blood cell count and differential blood cell count were determined according to the methods of Dacie and Lewis [17].

Statistical Analysis: One-way analysis of variance (ANOVA) was used to analyse the experimental data using Statistical Products and Service Solutions (SPSS) version 20. P <0.05 values were considered statistically significant and the data presented as mean ± standard deviation.

RESULTS

Percentage Yield of Ethanol Extract of P. Mutica Leaves:
The percentage yield obtained from the extraction of 500g of finely ground sample of P. mutica leaves was found to be 10.1%.

Quantitative Phytochemical Composition of P. mutica Leaves: The result presented in Table 1 reveals the relative abundance of important bioactive phytochemicals such as flavonoids, carotenoids, alkaloids and terpenoids in the plant extract.

Table 1: Quantitative Phytochemical Composition of P. mutica Leaves

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Composition (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>508.03 ± 6.93</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>802.91 ± 15.01</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>1361.66 ± 41.80</td>
</tr>
<tr>
<td>Saponin</td>
<td>51.82 ± 0.20</td>
</tr>
<tr>
<td>Resin</td>
<td>193.73 ± 0.50</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>93.32 ± 3.31</td>
</tr>
<tr>
<td>Steroids</td>
<td>61.78 ± 37.10</td>
</tr>
<tr>
<td>Tannins</td>
<td>10.23 ± 0.19</td>
</tr>
<tr>
<td>Phenols</td>
<td>129.88 ± 1.70</td>
</tr>
<tr>
<td>Cyanogenic glycoside</td>
<td>30.38 ± 0.27</td>
</tr>
<tr>
<td>Carotenoid</td>
<td>814.56 ± 8.18</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>658.86 ± 2.80</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (n = 3)

Effect of P. mutica Leaves on Percentage Parasitaemia of Malaria-Infected Mice: In Table 2, no parasitaemia was observed in the negative control (group 1) throughout the duration of the experiment. However, in established Plasmodium berghei infection, it was observed that there was a daily increase in percentage parasitaemia in the positive control group. However, there was a significant (p < 0.05), dose-dependent reduction in percentage parasitemia in the extract-treated groups when compared with the positive control as observed in day 2 and day 3 post-treatment. The decreases in percentage parasitaemia observed in the extract-treated groups were comparable to that of group 3 (treated with standard antimalarial drug).

Table 2: Percentage Parasitaemia of Malaria Parasite-Infected Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day one</th>
<th>Day two</th>
<th>Day three</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>0.00 ± 0.000</td>
<td>0.00 ± 0.000</td>
<td>0.00 ± 0.000</td>
</tr>
<tr>
<td>Group2</td>
<td>5.02 ± 0.5718</td>
<td>7.34 ± 0.9659</td>
<td>8.40 ± 0.7416</td>
</tr>
<tr>
<td>Group3</td>
<td>4.60 ± 1.1510</td>
<td>2.82 ± 0.7529</td>
<td>1.34 ± 0.4393</td>
</tr>
<tr>
<td>Group4</td>
<td>4.74 ± 1.0990</td>
<td>3.20 ± 0.4472</td>
<td>1.98 ± 0.5805</td>
</tr>
<tr>
<td>Group5</td>
<td>4.98 ± 2.5994</td>
<td>4.66 ± 0.5029</td>
<td>2.08 ± 0.5540</td>
</tr>
<tr>
<td>Group6</td>
<td>5.84 ± 0.4505</td>
<td>3.66 ± 0.5683</td>
<td>2.20 ± 0.4847</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (n = 5)

Effect of P. mutica Leaves on Packed Cell Volume (PCV) of Malaria Parasite-Infected Mice: In figure 1, the positive control exhibited significant (p < 0.05) decrease in PCV relative to the negative control. Malaria parasite-infected groups treated with varied doses of the ethanol extract as well as the negative control and standard control respectively showed significant (p < 0.05) increase in PCV relative to the positive control. There was no significant (p > 0.5) difference observed between the standard drug-treated group and ethanol extract treated groups.

Effect of P. mutica Leaves on Haemoglobin (Hb) Concentration of Malaria Parasite-Infected Mice: The group 2 (positive control) showed significant (p < 0.05) decrease in Hb concentration when compared with the negative control (group 1). The ethanol extract-treated groups as well as the standard control respectively showed significant (p < 0.05) increase in Hb concentrations when compared with the positive control, however, no significant (p > 0.05) difference in Hb concentrations was observed in the ethanol extract-treated groups when compared with the standard antimalarial drug-treated group.
Fig. 1: Packed Cell Volume (PCV) of Malaria Parasite-Infected Mice Treated with Ethanol Extract of *P. mutica* Leaves

Fig. 2: Haemoglobin (Hb) Concentrations of Malaria Parasite-Infected Mice Treated with Ethanol Extract of *P. mutica* Leaves

Fig. 3: Platelet count of Malaria Parasite-Infected Mice Treated with Ethanol Extract of *P. mutica* Leaves

Fig. 4: Red blood cell (RBC) count of Malaria Parasite-Infected Mice Treated with Ethanol Extract of *P. mutica* Leaves
Effect of *P. mutica* Leaves on Platelet count of Malaria Parasite-Infected Mice: The data in the figure 3 show no significant (p > 0.05) change in the platelet count of group 2 (positive control) when compared with the group 1 (negative) control. Also, there were no significant (p > 0.05) difference observed between the platelet counts of the standard antimalarial drug- treated mice (group 3) and ethanol extract- treated mice (groups 4, 5 and 6).

Effect of *P. mutica* Leaves on Red blood cell (RBC) Count of Malaria Parasite-Infected Mice: The result presented in figure 4 shows significant (p < 0.05) decrease in RBC count in group 2 (positive control) relative to group 1 (negative control). However, both the standard antimalarial drug- treated mice (group 3) and ethanol extract of *P. Mutica* -treated mice (groups 4 – 6) showed significant (p < 0.05) increase in RBC count when compared to the positive control but no significant (p > 0.05) difference was observed in RBC count of group 3 (treated with standard antimalarial drug) with respect to groups 4-6 respectively.

Effect of *P. mutica* Leaves on White blood cell (WBC) count of Malaria Parasite- Infected Mice: The data in the figure 5 reveal significant (p < 0.05) decrease in WBC count in group 2 (positive control) with respect to the treated groups. There was no significant (p > 0.05) difference observed in the WBC count in ethanol extract-treated groups with respect to the standard antimalarial drug- treated group as well as the negative control group, though both the ethanol extract-treated groups and the standard antimalarial drug- treated group showed significant (p < 0.05) increases in WBC counts when compared with the WBC count of the positive control.

Discussion

The study evaluated antimalarial activity and effect of ethanol extract of *P. mutica* leaves on haematological indices in *plasmodium berghei*-infected mice. Acute toxicity study of ethanol extract of *P. mutica* leaves showed this extract to have a median lethal dose (LD$_{50}$) > 5000 mg/kg/day, suggesting that it is relatively safe at the doses used in this study. Quantitative phytochemical analysis of the plant leaves revealed the presence of alkaloids and flavonoids in high concentrations; tannins and terpenoids in moderate concentrations, while saponins were present in low concentrations. Antimalarial activity evaluation showed that ethanol extract of *P. mutica* leaves evoked remarkable antimalarial activity. Mice infected with *plasmodium berghei* were characterized by parasitaemia which continued to rise steadily in untreated infected mice. This is in accordance with previous reports on antiplasmodial activities of medicinal plants [18-20]. *P. mutica* was able to significantly (p < 0.05) reduce the parasitaemia at the doses used in this study (in day 2 and day 3), indicating its antimalarial effect. The antimalarial activity exhibited by the plant extract was similar to that of the standard antimalarial drug, artemesunate. The mechanism of action of the leaf extract has not been evaluated in the present study, some of constituents detected such as alkaloids, flavonoids and tannins and terpenoids have been implicated in antiplasmodial activities [21-24].

In the haematological studies, the significant decrease in the packed cell volume (PCV) of the positive control with respect to the negative control may be due to haemolysis of malaria parasites- infected blood cells which might have occurred more rapidly than new blood cells are produced by the haematopoietic stem cells. The decrease in the PCV of malaria- infected but untreated
mice is in line with findings of Ovuakporaye [25] and Imoru et al. [26] who previously reported that changes in haematological parameters of malaria patients involve the major blood cells such as red blood cells, leucocytes and thrombocytes which are the major components of PCV. The significant increase in PCV observed in standard drug and extract- treated groups, respectively, show that the standard drug and the extract each could ameliorate the negative effect of malaria parasites on PCV in malaria-infected mice.

The significant decrease in haemoglobin (Hb) concentration observed in the positive control group with respect to the negative control group shows that malaria parasites depreciated the Hb concentrations of malaria-infected mice. The significant increase in Hb concentrations observed in standard drug and extract-treated groups respectively show that the standard drug and the extract each could ameliorate the negative effect of malaria parasites on Hb concentration in malaria-infected mice. The decreased Hb concentration in malaria parasite- infected but untreated mice recorded in this study is in agreement with the findings of George and Ewelike-Ezeani [27].

There was no significant change in the platelet count of the negative control (group 1) relative to the positive control (group 2). Also, there was no significant change in the platelet count observed in standard drug and extract- treated groups, respectively, with respect to the positive control (group 2). This observation may be due to the short duration of study. This is in agreement with previous findings which showed that platelet count is dependent on the parasite density [28].

Malaria parasite- infected but untreated mice (positive control) showed significant decrease in RBC count when compared to the negative control. However, both the standard drug and extract- treated mice, respectively, showed potentials in maintaining normal RBC counts of malaria parasite- infected mice. Anaemia is the major clinical sign and cause of mortality in animals with malaria infection where malaria parasites invade erythrocytes of infected animals, resulting in the destruction of the parasitized erythrocytes as a result of accelerated haemolysis [29] and increased phagocytosis of malaria-infected red blood cells. The anaemia recorded in this study (as shown by both the haemoglobin content and RBC of infected mice) may be attributed to increased haemolysis and phagocytosis of parasitized red blood cells. In this study, P. mutica was able to significantly increase the reduced number of erythrocytes as well as the haemoglobin content.

The malaria parasites- infected mice had significantly decreased white blood cell (WBC) count which could be attributed to the malaria parasites exhibiting destructive effects on the haematopoietic stem cells. It could also be that most WBC involved in fighting malaria parasites were destroyed faster than new ones were made. P. mutica was able to significantly increase the WBC of treated infected groups of mice with respect to the positive control group. White blood cells are first line defence mechanism against invading pathogens such as malaria parasites [30,31]. Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathogenesis. Malaria infected patients tended to have significantly lower platelets, WBCs, Lymphocytes, Eosinophils, RBCs and Hb levels in comparison to non-malaria infected patients [5,25]. In the present study the plant extract restored the altered haematological indices due to malaria parasite infection in mice. This is consistent with the findings of Fidele et al. [32] in Cameroon who identified flavonones, bergenins, chaalcones, rotenoids, isoflavones and phenolic contents of many plants like, Kegelia africana, Erythrina abyssinica, Moringa oleifera amongst many others as important active constituents of the plants extract which suppress Plasmodium falciparum growth in vitro.

CONCLUSION

The findings suggest that P. mutica leaves have antimalarial activity and ameliorative effects on altered haematological indices in malaria parasite-infected mice. This gives scientific evidence to the claims by traditional medicine practitioners that the plant is used in the management and treatment of malaria.

REFERENCES


