Antimalarial activity of methanolic extract of *Phytolacca dodecandra* leaves against *Plasmodium berghei* infected Swiss albino mice

Getinet Mequanint Adinew
Lecturer in Debremarkos University School of Medicine, Ethiopia.

ABSTRACT

Background: Malaria is the largest cause of death and disability globally and antimalarial drug resistances are a major public health problem, which hinders the control of malaria. Traditionally used medicinal plants have played important role in the treatment of malaria. In this study, the leaves of *Phytolacca dodecandra*, used in indigenous medicine to treat malaria in Ethiopia was evaluated for an *in vivo* antimalarial activity against chloroquine sensitive *Plasmodium berghei*. The objective of the study was to evaluate the antimalarial activity of methanolic leaf extract of *P. dodecandra* against *Plasmodium berghei* infected mice.

Materials and Methods: A total of 25 mice were randomly assigned into five groups with five mice per group. Three groups received the extracts at 100, 200 and 400 mg/kg body weight respectively. The other two groups (negative and positive control) were treated with 1ml/100 g body weight of Tween 80 and chloroquine at dose of 10 mg/kg respectively. Antimalarial activity was evaluated on day 4. Statistical analysis was done with ANOVA.

Results: The plant was nontoxic to mice and showed significantly improved suppression of parasitemia, prevented packed cell volume reduction (p < 0.05) dose dependently and increased the survival time of infected mice when compared to the negative control.

Conclusion: The leaves of *Phytolacca dodecandra* demonstrated antimalarial activity in mice.

Key words: Antimalarial activity, *Phytolacca dodecandra*, *in vivo*, *Plasmodium berghei*.

INTRODUCTION

Having been recorded as early as 1500 B.C, malaria is an ancient and one of the major fatal parasitic killer diseases of the world.[1] It is one of the most prevalent human infectious diseases worldwide. Greater than 40% World's population lives in malaria-endemic areas; 60% of malaria deaths worldwide occur in the poorest 20% of the population.[2] In Ethiopia, it is estimated that three-fourths of the land below 2000 meters is malarious with two-thirds of the country’s population at risk. It also accounts for “15% of admissions and 29% of inpatient deaths”.[3]

Antimalarial drug resistance is associated with increased incidence of morbidity and mortality,[4] and hence there is an urgent need to discover new compounds.[5] With high levels of transmission, malaria reduces gross domestic product by as much as 1.3% per annum and in Africa, malaria is now the fourth-leading cause of years of productive life lost which significantly slows economic growth and development.[6]

In most African countries including Ethiopia, medicinal plants have played important role in malaria treatment.[7] There is a wide
range of potentially useful medicinal plants available in Ethiopia and nearly 80% of the population in health care system uses this as remedy for ailments. In this study, the pharmacological activity of the leaf extracts from the traditionally used plant *Phytolacca dodecandra* was studied as there was no such study documented in the literature.

**MATERIALS AND METHODS**

**Plant material and extraction**

The plant *Phytolacca dodecandra* was collected in Gondar town of Ethiopia. It was identified by Dr Samuel Sahilea, a Botanist from Department of Biology in University of Gondar (UoG). The leaves were dried at room temperature and milled. 164 g of the powdered leaves was soaked with 80% methanol for 72 h. The extracts were concentrated in dry hot oven. The percentage yield was 18.04 g (11%). The dry extract (PDME) was stored in a refrigerator at -4°C until used to ensure the apparent stability and activity of the PDME.

**Phytochemical screening**

Phytochemical screening of the plant was performed according to the standard screening methods. The PDME was screened for bioactive ingredients such as saponins, flavonoids, anthraquinones, alkaloids, tannins, phenols, sterols, resin, terpinoids and cardiac glycosides.

**Animals**

Swiss albino mice of either sex, 7–8 weeks old were obtained from the Laboratory Animal Centre of the pharmacology department, UoG. All animals were handled according to the international guideline for animal welfare. Permission and approval for animal studies were obtained from department of pharmacology, UoG.

**Acute toxicity study**

Acute oral toxicity of PDME was studied according to the Organization for Economic Co-operation and Development (OECD) guideline No 420 “Acute oral toxicity – fixed dose procedure”. One female and one male mouse were administered at 2,000 mg/kg, if these survived; four additional mice were sequentially dosed at approximately 48 hour intervals. A total of five female and five male mice were tested. The mice were fast 4 hours prior to administration and returned to feeding 1 hour later. On the day of PDME administration, all the mice were observed for mortality and signs of toxicity at 30 min, 4 and 24 hours following administration and thereafter twice a day for 14 days.

**Micro-organisms and inoculum preparation**

Fourth day (D-4) suppressive activity of the PDME was assessed using the method described by Okokon JE, et al. A strain of *Plasmodium berghei* (P. berghei) which is chloroquine sensitive was obtained from the department of Pharmacology, UoG. A donor mouse with a rising parasitemia of 30% with the *P. berghei* was used for inoculum preparation. The desired blood volume was drawn from the donor mice and diluted serially in normal saline (0.9 %) to contain about $1 \times 10^7$ infected RBC’s in every 0.2 mL suspension.

On the first day (D0), the 0.2 mL suspension was injected using a 1 ml syringe and ½ inch 23-gauge needle into mice intraperitoneally to initiate infection. The inoculated animals were then randomized into five groups of five mice per cage and maintained in the Animal Room, Department of Pharmacology, in accordance with the Internationally accepted principles for laboratory animals’ use and care.

**Study design and treatments**

A total of 25 *P. berghei* inoculated mice were randomly assigned into five groups with five mice per group. After 2 hours post-infection, the experimental groups were treated orally with 100, 200 and 400 mg/kg/day doses of the PDME. The reference group was treated with chloroquine (10 mg/kg) and the control group received Tween 80 of 2% 10 mL/100 gm body weight. All were repeated for the next 3
days (D₁ to D₃). Totally animals received the drug for 4 days.

**Determination of body weight**

The body weights of the mice were measured to observe whether the test of the PDME prevents body weight loss. The weights were taken on day 0 and D - 4.

**Peripheral smear test for parasitaemia**[20]

After four days of treatment, on 5th day, thin blood smears were made from the tail of each mouse. The blood films were fixed with methanol, stained with 10% Giemsa for 10 min and parasitaemia examined microscopically. The parasitaemia level was determined by counting the number of parasitized erythrocytes out of 100 erythrocytes in random fields of microscope. Average parasitaemia and chemosuppression was calculated as follows;

\[
\% \text{ Parasitaemia} = \frac{\text{No. of parasitized RBC} \times 100}{\text{Total No. of RBC counted}}
\]

Average percentage of chemosuppression was;

\[
= \frac{A-B \times 100}{A}
\]

Where, A is the average percentage parasitaemia in negative control group and B, average percentage parasitaemia in the test group.

**Determination of packed cell volume**

Packed cell volume (PCV) is a measure of the proportion of red blood cells (RBC) to total blood volume, used in estimating the mean erythrocyte hemoglobin concentration. The PCV was measured to predict the effectiveness of the PDME. Blood from the tail of the animals was drawn up to a ¾ of the 100 mm mark microhaematocrit tube. The tube was then sealed by sealant at both ends and centrifuged for 5 minutes about 5000 rpm. The PCV of each mouse was then measured before infection and on day 4 after infection using the formula;

\[
= \frac{\text{volume of RBC in a given volume of blood} \times 100}{\text{Total volume of blood examined}}
\]

**Determination of mean survival time**

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow up period. The mean survival time (MST) for each group was calculated as follows;

\[
= \frac{\text{sum of survival days of all mice in a group}}{\text{Total number of mice in that group}}
\]

**Statistical analysis**

Results of the study were presented as mean ± SEM. Data were analyzed using SPSS version 20. Statistical significance was undertaken by ANOVA tests coupled to LSD to compare result between doses and among treatment and control groups. Mean PCV and body weight before and after infection and treatment were compared by two-tailed paired t-test. The result was considered statistically significant at 95% confidence level and P-value < 0.05.

### RESULTS

#### Toxicity test

According to the OECD guideline, the PDME did not produce any sign of toxicity during the first 30 minutes, 4 hrs and 24 hrs. No death was observed up to 14 days.

#### Phytochemical screening

Phytochemical screening of PDME revealed the presence of tannin, flavonoids, phenols, saponins, alkaloids, terpenes and steroids.

#### Changes in body weight

The PDME showed a dose dependent prevention of loss of body weight when compared to the negative control (Table 1).

#### In vivo antimalarial assays (4-day suppressive activity)

The extract caused a significant chemosuppression activity when compared to the negative control except the lower dose (100 mg/kg/day). The standard drug, chloroquine caused...
chemosuppression of 100%, which was more significant as compared to the extract treated groups (table 1).

**Packed cell volume**

In the present study, PCV values appear to improve with the extract on day 4 of treatment (table 2).

**Mean Survival Time**

The mice treated with the extract survived significantly longer than the mice in the negative control group. But parasitaemia increased gradually in all groups, and all the mice died on different days (table 2).

**DISCUSSION**

Methanolic extract of the *P. dodecandra* leaf was not toxic up to 2000 mg/kg body weight in mice. Changes in general behaviors, variations in body weight and mortality are critical for the evaluation of the effect of a compound on mice, since such changes are often the first signs of toxicity.\[^{[21]}\] The oral dose of >2000 mg/kg body weight is 10 times greater than the minimum effective dose of 200 mg/kg. Earlier reports have shown that if the median lethal dose of a test substance is three times more than the minimum effective dose, the extract is considered as safe\[^{[22]}\] and this could explain the safe use of the plant for the treatment of malaria by local people in Ethiopia.

The average parasitaemia of the extract revealed lower antimalarial activity, which had 56.2% lower potency than chloroquine phosphate. Similar finding with other plant extracts was reported by researchers in Ethiopia.\[^{[23]}\]

**Table 1: Effect of Phytolacca dodecandra leaf extract on body weight, Parasitaemia and Chemosuppression in Plasmodium berghei infected Swiss albino mice**

<table>
<thead>
<tr>
<th>Group, treatment</th>
<th>weight on Day 0 (gm)</th>
<th>weight on Day 4 (gm)</th>
<th>change of weight (gm)</th>
<th>Parasitaemia (%)</th>
<th>Chemosuppression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PDME 100 mg/kg</td>
<td>34.81 ± 3.11</td>
<td>30.43 ± 0.40</td>
<td>- 6.7[^{†}]</td>
<td>61.00 ± 0.03</td>
<td>18.67</td>
</tr>
<tr>
<td>2. PDME 200 mg/kg</td>
<td>32.34 ± 2.73</td>
<td>29.60 ± 1.87</td>
<td>- 4.42[^{*}]</td>
<td>36.80 ± 0.08[^{‡}]</td>
<td>50.93[^{‡}]</td>
</tr>
<tr>
<td>3. PDME 400 mg/kg</td>
<td>26.28 ± 1.61</td>
<td>24.90 ± 1.80</td>
<td>- 2.70[^{†}]</td>
<td>33.60 ± 0.10[^{‡}]</td>
<td>55.24[^{‡}]</td>
</tr>
<tr>
<td>4. Tween-80 of 2%</td>
<td>35.61 ± 1.49</td>
<td>30.33 ± 1.48</td>
<td>- 5.28[^{‡}]</td>
<td>75.00 ± 0.04</td>
<td>-</td>
</tr>
<tr>
<td>5. chloroquine 20mg/kg</td>
<td>30.62 ± 1.87</td>
<td>32.80 ± 1.14</td>
<td>+ 8.00[^{‡}]</td>
<td>0.00[^{‡}]</td>
<td>100[^{‡}]</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, PDME - *Phytolacca dodecandra* leaf methanol extract, \[^{*}\]p < 0.01, \[^{†}\]p < 0.001 paired t test within same group between day 0 and 4, \[^{‡}\]p < 0.05 vs. control, \[^{‡}\]p < 0.001 vs. control.

**Table 2: Effect of Phytolacca dodecandra leaf extract on on the packed cell volume (PCV) and mean survival time (MST) of mice in Plasmodium berghei infected Swiss albino mice**

<table>
<thead>
<tr>
<th>Group, treatment</th>
<th>PCV at Day 0</th>
<th>PCV at Day 4</th>
<th>PCV change</th>
<th>MST in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PDME 100 mg/kg</td>
<td>54.03 ± 0.48</td>
<td>47.62 ± 1.13</td>
<td>6.41[^{†}]</td>
<td>8.80 ± 0.97</td>
</tr>
<tr>
<td>2. PDME 200 mg/kg</td>
<td>52.66 ± 0.41</td>
<td>47.53 ± 0.92</td>
<td>5.13[^{†}]</td>
<td>10.00 ± 1.10</td>
</tr>
<tr>
<td>3. PDME 400 mg/kg</td>
<td>52.42 ± 0.28</td>
<td>48.38 ± 0.31</td>
<td>4.04[^{‡}]</td>
<td>14.20 ± 2.40</td>
</tr>
<tr>
<td>4. Tween-80 of 2%</td>
<td>50.81 ± 0.92</td>
<td>32.47 ± 0.92</td>
<td>11.38[^{‡}]</td>
<td>6.60 ± 0.24</td>
</tr>
<tr>
<td>5. Chloroquine 20mg/kg</td>
<td>51.47 ± 1.12</td>
<td>48.47 ± 0.44</td>
<td>3.00[^{‡}]</td>
<td>21.40 ± 2.27</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, PDME - *Phytolacca dodecandra* leaf methanol extract, \[^{*}\]p < 0.01, \[^{†}\]p < 0.001 paired t test within same group between day 0 and 4, \[^{‡}\]p < 0.05 vs. control, \[^{‡}\]p < 0.001 vs. control.
The extract of *P. dodecandra* had a good antiplasmoidal activity. Literature grades antimalarial activity of a compound as moderate, good and very good, if it displayed a percent growth inhibition of $\geq 50\%$ at a dose of 500, 250 and 100 mg/kg/day respectively.\(^{[24]}\) Even though the rodent malaria model, *P. berghei*, is not exactly similar to that of the human *Plasmodium* parasites, it is the first step to screen most of the *in vivo* antimalarial activities of new molecules and new therapeutics.\(^{[21]}\)

Biological activity of this plant could be attributed to the presence of various secondary metabolites and it may be acting singly or in synergy with one another to exert the observed antiplasmoidal activity of *P. dodecandra*. Not only their presence, but also the quantity of the metabolites in a given plant would determine the extent of its bioactivity. In addition, presence of $> 1$ class of secondary metabolites in a given plant extract determines the nature and extent of its biological activity.\(^{[25]}\)

The haematological findings showed that the PCV levels decreased after infection with *P. berghei*. This is an indication of anaemia, due to haemolysis, which is a consistent finding in blood protozoan parasites infection. The improvement in haematological parameters noticed in this study in a dose dependent manner may be due to the inhibitory effect of the extract and chloroquine on the plasmodium organisms, since the increase in the blood parameters corresponded to the decreased parasites load.\(^{[26]}\)

Studies showed that PCV on rodent malaria fell down to 43-44%, which occurred approximately 48 hours post-infection.\(^{[27]}\)

Malaria fever is a disease that is characterized by loss of appetite which ultimately leads to weight loss.\(^{[28]}\) In this study, we measured the body weights of the infected mice to monitor the effects of infection on factors such as food and water intake. The decrease in body weight of malarial mice was clearly evident from the 4$^{th}$ day of infection, and presumably due in part to the decrease in food intake. Decrease in body weight may also be the consequences of disturbed metabolic function and hypoglycaemia that has been reported to be associated with malaria infection.\(^{[29]}\)

The mice treated with the extracts had a survival time ranging from $8.8 \pm 0.97$ to $14.2 \pm 2.39$ days, while the corresponding value of the untreated control group was $6.60 \pm 0.24$ days, which is similar with other studies conducted by kalra et al.\(^{[30]}\)

Groups treated with chloroquine, though showed undetectable level of parasitaemia, all the mice died in 21.4 days. Similar findings were reported by Belay and Mengiste.\(^{[27,31]}\)

In several cases, remarkable suppression of parasitaemia by extracts translated into either a higher and/or a longer mouse survival. The methanolic extract of the leaves of *P. dodecandra* at a dose of 400 mg/kg had only 40% survival of mice on day 19 even though it had a significant parasitaemia suppression ($p < 0.05$). This may suggest that the bioactive compound in the plant may have a short half-life. Some antimalarial drugs including artemisinin-based derivatives are known to be fast acting, and to have a short half-life.\(^{[32]}\)

To conclude, the leaves of *Phytolacca dodecandra* showed antimalarial activity in mice which provides a base for further studies on this plant. The isolation and characterization of the bioactive principles from the plant may lead to the discovery of novel antimalarial compounds in near future.

**ACKNOWLEDGEMENT**

Authors thank Dr. Mohammedbirhan Abdulwahib, Assistant Professor for valuable suggestions.

**REFERENCES**


