In vivo Anti-malarial Activity of Methanol Root Extract of Securidaca longepedunculata in Mice Infected with Plasmodium berghei

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Abstract: Malaria is a major public health problem globally, and the alarming spread of drug resistance and limited number of effective drugs, underline how important it is to discover new anti-malarials. This study aims at investigating the in vivo anti-malarial activity of methanol roots extract of Securidaca longepedunculata against chloroquine (CQ)-sensitive (NK65) strain of Plasmodium berghei, inoculated into Swiss albino mice with an infection of about 1x10^7 parasites intra-peritoneally. Daily administration of the extract, for four days starting from the day of parasite inoculation; with the negative control received the same amount of normal saline which was used to suspend each dose of the herbal drug. Chloroquine (10 mg/kg) and artiemether (1.6 mg/kg) were used as standard drugs, and were administered through the same route intra-peritoneally. The extract was observed to suppress the Plasmodium berghei parasites in Swiss albino mice by 82.6%, 62.7% and 58.4% at doses of 0.56 mg/kg, 0.28 mg/kg, and 0.14 mg/kg while chloroquine (10mg/kg) and artiemether (1.6mg/kg) produced 93.8% and 84.4% respectively. The methanol root extracts promises to be a potent anti-marial; hence further work is recommended using different animal models.

Key words: Extract; malaria treatment; medicinal plants; Mice; Plasmodium berghei; parasite;
1. Introduction

Malaria is an infectious disease caused by the parasite plasmodia. Four different species of these cause different types of human malaria, namely *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium ovale* and *Plasmodium malariae* all of which are transmitted by the female anopheles mosquito. *Plasmodium berghei* is a laboratory animal species which data may be extrapolated to human species. Malaria is the worldwide most important parasitic disease with an incidence of almost 300 million clinical cases and over 1 million deaths per year (WHO, 1999). Almost 90% of these deaths occur in sub-Saharan Africa where young children are the most affected (Ouatara et al. 2006). Malaria is directly responsible for one in five childhood deaths in Africa and directly contributes to illness and deaths from respiratory infections, diarrheal disease and malnutrition (WHO, 1999).

In the absence of effective malaria vaccines, effective chemotherapy remains the mainstay of malaria control (Winstanley, 2000). The potentially lethal malaria parasites have shown themselves capable of developing resistance to nearly all used anti-malarial drugs (WHO, 1999) and resistance strains have rapid extension (Plowe et al. 1995). For obvious reasons malaria will continue to cause morbidity and mortality on a large scale in tropical and sub-tropical countries, the alarming rise at which the parasites (particularly *Plasmodium falciparum*) have developed resistance to currently used anti-malarial drugs makes it imperative to search for newer, more effective therapeutic agents. The loss of effectiveness of chemotherapy constitutes the greatest threat to the control of malaria. Therefore, to overcome malaria, new knowledge, products and tools are urgently needed; especially new drugs (Omulokoli et al. 1997, Rasoanaivo et al. 1992). The anti-malarial potentials of compounds derived from plants is proven by examples such as quinine, obtained from *cinchoma* species and *artemisinins* obtained from *artemesia species*. Traditional methods of malaria treatment could be a promising source of new anti-malarial compounds. In Africa, more than 80% of people use traditional medicines and most families have recourse to this medicine based on plants extracts for the curative treatment of malaria (Wright and Phillipson, 1990). In fact, the traditional medicine of this continent constitutes an important source for ethno pharmacological investigations.

The selection of plants to be screened for anti-malarial activity is done on the basis of traditional reputation of the plants for efficacy in the treatment of malaria e.g. It is reported that almost all parts of *S. longipedunculata* (leaves, twigs, stem, bark, root and seeds) are used by man for different purposes; the root and the bark are taken orally either powdered or as infusion for treating chest complaints, inflammation, abortion, ritual suicide, tuberculosis, infertility, venereal diseases and for constipation. Tooth ache can also be relieved by chewing the roots. Powdered roots are used to treat head ache by rubbing them on the fore head. Infusions of the roots are used for washing topical ulcers. In Limpopo the Venda people take the roots for mental disorders and against children’s illness.
during breast feeding. They also mix the powdered roots with maize and sorghum beverages for men being sexually weak. In Zimbabwe, the roots are given to people who are believed to be possessed by evil spirits. The root extracts are also used for menstrual pains and gonorrhea in Nigeria (Akiniyi and Sultanbawa, 1986) Powdered with salt and water they are used against snake bites and cough. They are also used as fish poison, molluscicide, snake repellent, or as insect repellent, source of fibre, water purifying agent, as an ornamental (Van Wyk, B-E. and Gericke, N. 2000., Neuwinger, 1996., Heywood, 1995). The aqueous extract of the root/leaf has high concentrations of glycosides, saponins and tannins and so may be responsible for some of these activities. The root extracts are also used for menstrual pains and gonorrhoea in Nigeria (Akiniyi and Sultanbawa, 1986). The claims of the medicinal application of the root of this plant are numerous. The aim of this study was to investigate the in-vivo anti-malarial activity of methanol extract of *S. longepedunculata* on *P. berghei*, using laboratory animal species which data may be extrapolated to human species.

2. Materials and Methods

2.1. Plant Material

The roots of *S. longepedunculata* were collected from Zuru a town in Kebbi State, Northern Nigeria. The plant was authenticated by a taxonomist at the herbarium section of Biological Sciences Department, Ahmadu Bello University Zaria, Nigeria. Where a voucher specimen was deposited and it was given a voucher number: 900149. The roots were thoroughly washed with water, air dried and pounded into powder using pestle and mortar.

2.2. Extract Preparation

The methanol crude extracts of the roots of *S. longepedunculata* was obtained by Soxlet extraction using methanol for 48 hours. The extract was concentrated to dryness on a water bath between 40-45°C. The extracts were stored in sealed bottles at room temperature until tested.

2.3. Parasite Strain

The *plasmodium* species used in this work was that which is mostly employed in rodent malaria parasite- *Plasmodium berghei* NK65 chloroquine sensitive strain, and was obtained from Biochemistry Department, Ahmadu Bello University, Zaria, Nigeria.

2.4. Animals and Animal Husbandry

Healthy adult male Swiss albino mice weighing approximately ± 21.166 g were used for this study; the animals were obtained from the Faculty of Pharmaceutical Sciences, Ahmadu Bello
University, Zaria, Nigeria. They were housed in cages at the same Departmental animal house, fed on standard mouse feed (vital feeds, Jos, Nigeria) and allowed access to water ad libitum. The animals were allowed to acclimatize to the laboratory conditions for at least 3 days before being subjected to the experiments. All the experiments were carried out in a conducive laboratory setting that has ambient illumination and a temperature that is close to that of the animal house, according to OECD guidelines.

2.5. The Anti-malarial Tests

Tests were performed using a four day curative standard test (David et al. 2004., Peter and Anatoli, 1998., WHO, 1980) and employing chloroquine sensitive *P. berghei* NK 65 strain.

The mice were divided weight dependently into 6 groups of five each, consisting of three methanol extracts/treatment groups; 5%, 10%, and 20% of the extract’s LD$_{50}$ equivalent to 0.14, 0.28, and 0.58 mg/kg, chloroquine standard control 10 mg/kg, artemether standard control group 1.6mg/kg, infected but not treated negative control group. All the groups were infected with the malaria parasites- *P. berghei* (chloroquine sensitive strain) for a four day suppressive test. The *P. berghei* was subsequently maintained in the laboratory by serial blood passage from mouse to mouse.

For this study, a donor mouse with a rising parasitemia of 20% was sacrificed and its blood was collected (in a slightly heparinized syringe) and the blood was diluted with Trisodium citrate (TC) medium so that each 0.2 ml contained approximately 1X10$^7$ infected red blood cells (David et al. 2004, Peter and Antoli, 1998). Invariably each animal received inoculums of about 1X10$^7$ parasites per kilogram body weight through needle passage and it produced infection in the mice.

On commencement of the 4- day suppressive standard test, 3 hours after infecting the mice with the malaria parasites, the methanol root extract of *S. longipedunculata* was administered to the three test groups in divided doses of 0.14 mg/kg, 0.28 mg/kg, and 0.56 mg/kg of the plant’s LD$_{50}$, chloroquine and artemether were given to the standard groups at the doses of 10.00 and 1.60 mg/kg respectively, daily for 4 consecutive days, i.e. from Day 0 to Day 3 (David et al 2004). The negative control group received no medication but was infected. All the drugs were given through intra-peritoneal routes.

2.6. Estimation of Parasitemia

Thin smears of blood films were obtained from the tail end of each mouse on day 4 after infection and treatments (David et al. 2004 and WHO, 1980). The smears were placed on microscopic slides, fixed with methanol and stained with 10% Giemsa at pH 7.2 for 15 minutes, and examined under the microscope at X100 magnification to assess the level of parasitemia. The percentage
parasitemia was calculated according to the method outlined by Iwalewa et al. (1997) as percentage parasitemia = (No. of parasites in treated /No. of parasites in control X100.

2.7. Determination of LD$_{50}$

The method of Locke (1983) was modified and used to determine the dose of the extract that would be lethal to 50% of the population of the animals. Three dose points were (10, 100 & 1000 mg/kg) were chosen for the pilot experiment, from which doses of 1, 2, 4 and 8 mg/kg respectively were given to one animal per group in the second phase intra-peritoneal. The geometric mean of the highest non-lethal dose and the lowest lethal dose was used to calculate the LD$_{50}$ Figures 1 and 2

2.8. Statistical Analysis

All data were expressed as the means ± standard error of the mean (SEM), one way analysis of variance (ANOVA) with subsequent dunnett’s post-hoc analysis was used to detect further differences between groups using SPSS 16.0 version. Differences were considered statistically significant at P<0.05.

3. Results and Discussion

The results of this study reveal that in vivo methanol extracts of S. longepedunculata displayed very good activities against P. berghei malaria parasite. The dose dependent administration of the extracts indicates that 20% of the LD$_{50}$ (0.56 mg/kg) showed a statistically significant difference (P< 0.05) on day 4 parasites level compared to the negative control group; i.e. 2.8 ± 0.80 : 16.1± 3.0 respectively (table 1). The 10% of LD$_{50}$ (0.28 mg/kg) equally showed a statistically significant difference (P< 0.05) when compared to the negative control; i.e. 6.0 ± 0.57: 16.1 ± 3.0 (table 1). Likewise, the 5% LD$_{50}$ of the extract (0.14 mg/kg) also showed a statistically significant difference (P< 0.05) when compared to the negative control group; i.e. 6.7 ± 0.34: 16.1 ± 3.0 (Table 1).

In the whole of this study, the highest level of clearance was observed in chloroquine (10 mg/kg) treated group, with percentage parasitemia on day 4 as 1.0 ± 0.40 as compared to 16.1 ± 3.0 of the negative control group (table 1). Artemether 1.6mg/kg equally showed good clearance of parasitemia on day 4, but there is no significant difference (P> 0.05) between artemether 1.6 mg/kg and 0.56 mg/kg of S. longepedunculata methanol root extracts (table 2). The analysis of the Packed Cell Volume (PCV) on day 4 indicated that all the extracts prevented the reduction in PCV significantly (P< 0.05) compared to the negative control (table 2). Results of this work also reveal that the group of mice given 0.56 mg/kg of the extracts indicated that the extracts did not significantly prevented weight loss (P< 0.05) compared to the negative control group, and the increase in body weight was not dose
dependent related (table 3), all these could be associated to the presence of the metabolites found in the plant (Table 4).

### Table 1. Parasitemia suppressive test of methanol root extracts of *Securidaca longepedunculata* against *Plasmodium berghei* in mice after 4 days

<table>
<thead>
<tr>
<th>Drug / Dose (mg/kg)</th>
<th>% Parasitaemia*</th>
<th>% Suppression*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (0)</td>
<td>16.1 ± 3.00</td>
<td>-</td>
</tr>
<tr>
<td><em>S.l.</em> (0.14)</td>
<td>6.7 ± 0.34</td>
<td>58.4</td>
</tr>
<tr>
<td><em>S.l.</em> (0.28)</td>
<td>6.0 ± 0.57</td>
<td>62.7</td>
</tr>
<tr>
<td><em>S.l.</em> (0.56)</td>
<td>2.8 ± 0.80</td>
<td>82.6</td>
</tr>
<tr>
<td>Chloroquine (10.00)</td>
<td>0.9 ± 0.40</td>
<td>93.8</td>
</tr>
<tr>
<td>Artemether (1.60)</td>
<td>2.5 ± 0.60</td>
<td>84.4</td>
</tr>
</tbody>
</table>

*S.l.* = *Securidaca longepedunculata*; N = 5 per group; *Data are means ± SEM.

### 3.1. Packed Cell Volume (PCV)

PCV is a measure of the proportion of red blood cells to plasma. This test was carried on each mouse just before the infection and on the 5th day of infection. Table 2 shows the PCV values.

### Table 2. PCV values in mice infected with *P. berghei* and treated with methanol root extracts of *Securidaca longepedunculata*

<table>
<thead>
<tr>
<th>Drug / Dose (mg/kg)</th>
<th>PCV (%)*</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D0</td>
<td>D4</td>
</tr>
<tr>
<td>Normal saline (0)</td>
<td>53.75 ± 0.95</td>
<td>48.33 ± 1.22</td>
</tr>
<tr>
<td><em>S.l.</em> (0.14)</td>
<td>52.75 ± 1.70</td>
<td>52.00 ± 1.47</td>
</tr>
<tr>
<td><em>S.l.</em> (0.28)</td>
<td>56.33 ± 1.22</td>
<td>55.66 ± 2.35</td>
</tr>
<tr>
<td><em>S.l.</em> (0.56)</td>
<td>57.00 ± 1.80</td>
<td>56.50 ± 0.51</td>
</tr>
<tr>
<td>Chloroquine (10.00)</td>
<td>52.75 ± 1.70</td>
<td>48.5 ± 1.19</td>
</tr>
<tr>
<td>Artemether (1.60)</td>
<td>53.30 ± 0.89</td>
<td>49.00 ± 3.08</td>
</tr>
</tbody>
</table>

*S.l.* = *Securidaca longepedunculata*; N = 5 per group; *Data are means ± SEM.
Table 3. Body weight of *P. berghei* infected mice after treatment with methanol root extracts of *Securidaca longepedunculata*

<table>
<thead>
<tr>
<th>Drug / Dose (mg/kg)</th>
<th>Weight (g)*</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline (0)</td>
<td>23.0 ± 0.89</td>
<td>23.4 ± 0.75</td>
</tr>
<tr>
<td><em>S.l.</em> (0.14)</td>
<td>22.0 ± 0.83</td>
<td>23.2 ± 1.16</td>
</tr>
<tr>
<td><em>S.l.</em> (0.28)</td>
<td>18.4 ± 1.36</td>
<td>18.4 ± 1.43</td>
</tr>
<tr>
<td><em>S.l.</em> (0.56)</td>
<td>19.4 ± 0.75</td>
<td>19.0 ± 0.83</td>
</tr>
<tr>
<td>Chloroquine (10.00)</td>
<td>21.6 ± 0.93</td>
<td>23.0 ± 1.34</td>
</tr>
<tr>
<td>Artemether (1.60)</td>
<td>22.6 ± 1.60</td>
<td>22.4 ± 1.32</td>
</tr>
</tbody>
</table>

*S.l.* = *Securidaca longepedunculata*; N = 5 per group; *Data are means ± SEM.

The phytochemical screening of *S. longepedunculata* components was determined using the method of Sofowora (1993) and Harbone (1973) with little modifications. The percentage yield of the methanol roots extract is gotten from the relation bellow:

\[
\text{Percentage yield} = \frac{\text{Weight of sample extract obtained (g)}}{\text{Weight of powder sample used (g)}} \times 100 \% = \frac{101.8 \times 100\%}{200} = 50.9\%
\]

The data are show in table 4.

Table 4. Results of phytochemical screening of the methanolic root extract of *S.longepedunculat*

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>-</td>
</tr>
</tbody>
</table>

The selection of this plant for *in vivo* anti-malarial activity is on the basis of its established anti protozoan results/effects both *in vitro* and *in vivo* (Atawodi et al. 2002., Ameh et al.2007., Aderbauer et al., 2008., Yusuf et al. 2008). The 4- day suppressive test is a standard test commonly carried out for
anti-malarial screening, and the determination of percentage of inhibition of parasitemia is the most reliable parameter. A mean group parasitemia level of less than or equal to 90% that of mock treated control animals usually indicates that the test compound is active in standard screening studies (Peter and Antoli, 1998). Therefore, from the results of this work (table 1) the methanol root extracts of *S. logopedunculata* on *P. berghei* infected in mice, the percentage of parasitemia measured changed significantly from those in the control animals. This significant suppression of parasitemia by the methanol extract of *S. longepedunculata* in divided doses on day 4 is in agreement with (Oketch-Rabah, 2003), and also suggestive that the phytochemistry of *S. longepedunculata* which appears to be dominated by saponins (table 4) that are capable of acting as detergents to assist in the dissolution of active ingredients in water, while flavonoids, methyl salicylate, 3,4,5-o- caffeoyl quinic acid, securine, fatty acids, triacylglycerols and other monosaccharides (table 4) might be able to potentiate or be responsible for the antiprotozoan/ antibacterial activity of *S. longepedunculata* in crude preparations (Sapeika, N, 1944 and Meli et al. 2007).

From the results in table 1, the two standards anti-malarial drugs employed in this study; chloroquine and artemether as controls 10 mg/kg and 1.6 mg/kg respectively, which showed percentage parasitemia suppressions of 93.8% and 84.4% respectively after 4 days treatment did not show significant difference (P> 0.05) in comparison with that from the percentage parasitemia suppression exhibited by the methanol root extract of *S. longepedunculata*; 82.6%.

In other words, after the 4- day’s treatment of mice infected with *P. berghei* with chloroquine 10 mg/kg, artemether 1.6 mg/kg, *S. longepedunculata* root extract 0.56 mg/kg the percentage parasitemia as shown by the slides respectively: 1.0 ± 0.4, 2.5 ± 0.6, 2.8 ± 0.8, revealed that there is a significant difference (P< 0.05) between all the three drugs and the negative control group with 16.1 ± 3.0 as its percentage parasitemia in 4 days (table 1).

It was observed from these results that in the untreated group, there was a significant increase in the parasite count (P< 0.05) compared to the treated groups, and the hematocrit packed cell volume (PCV) decreased markedly from day -0 to day -4 (table 2). This is in agreement with previous studies (Ayodele, 1979). Meanwhile, the extracts treated groups did not show a significant decrease in PCV (P> 0.05) when compared to the negative control group. This is suggestive that the extract may contain some substances that either increase appetite or blood quality to the animals, in addition to its anti-parasitic activity (Abebe et al. 2003).

**4. Conclusion**

From the present study, it can be concluded that the methanol roots extract of *S. longepedunculata* have shown potent parasite suppressive effects on *P. berghei* infected Swiss albino...
mice in a dose related fashion, has also maintained the PCV of the infected animals even more than the chloroquine and artemether; standard drugs, as well as the weights of the infected animals. Hence, more work needs to be done on this plant to uncover its potencies.

References


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