



## Tolerance and Antiplasmodial Screening of *Ritchea longipedicellata* in *Plasmodium berghei*

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### Abstract

The tolerance and antiplasmodial activity of methanolic root extract of *R. longipedicellata* in *P. berghei* infected mice was investigated. Extract was administered to mice at 1500mg/kg for 30days and liver and kidney parameters were analysed. Mice were infected with *P. berghei* and administered the extract and reference drugs 2hrs and 5days post-infection for suppressive and therapeutic activities respectively. At 1500mg/kg dose, *R. longipedicellata* extract exhibited a significant decrease ( $p \leq 0.05$ ) in ALP, GOT, GPT and Creatinine. Bilirubin showed no significant change while PCV was increased ( $p \leq 0.05$ ). Inhibition in suppressive activity at 50 and 100mg/kg doses of the extracts were 86.8% and 65.43% while artesunate (120mg/kg) and chloroquine (8mg/kg) were 100%. Clearance rate in therapeutic activity for 50 and 100mg/kg dose of *R. longipedicellata* extract were 36.73% and 64.60%, lower than chloroquine (80.85%) and artesunate (100%). Longest survival period was observed in 50mg/kg suppressive group than all the groups treated with *R. longipedicellata* methanolic root extract. This study suggests that the methanolic root extract of *R. longipedicellata* is well tolerated and possesses antiplasmodial activity in mice infected with *P.berghei*.

**Keywords:** *Ritchea longipedicellata*, *plasmodium berghei*, tolerance, suppressive, therapeutic

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## INTRODUCTION

Herbal medicines still remains the major source of health care delivery in Africa. Establishing the safety and efficacy of these herbal medicines pose a major challenge to the African medical scientist.

In Africa, the malaria situation has deteriorated during the past decades<sup>1</sup> for several reasons, one of which is the emergence of drug resistance strain of plasmodium. This has compromised malaria therapy and has led to the search for new lead compounds in medicinal plants used in folk medicine for the treatment of the disease. This is predicated on the fact that studies have shown a high correlation between pharmacological activity/clinical use of plant isolates and their established use as herbal medicine<sup>2,3,4</sup>. Although aqueous decoction is the most common method of ethno medical preparation, alcoholic extraction is also widely used especially in the Delta region of Nigeria. The roots or stem or sometimes leaves of these plants are cut in bits and put into bottles into which locally distilled ethanol is added. This preparation is dispensed in a shot glass every morning to prevent malaria. In Africa, traditional healing methods with herbs take a holistic approach to health care. The use and action of the whole plant is preferred to isolated constituents of the plant. Some workers report that this approach produces synergy<sup>5,6</sup>. However, most of these plants have not been investigated for safety or efficacy. Therefore the therapeutic values of these plants need to be supported by scientific data of their safety and efficacy.

Thus, the objective of this study is to evaluate the tolerance level and the antiplasmodial activity of methanolic root extract of *R.longipedicellata* Gilg (Capparidaceae) in *Plasmodium berghei* infection in mice. *R longipedicellata*, a climbing shrub is used as a tonic and antimalaria in herbal medicine in Nigeria.

## MATERIALS AND METHOD

### Chemicals and Solvents

All solvents used for extraction were of analytical grade and supplied by Sigma-Aldrich

Chemical Company Ltd. Germany. Bioassay of biochemical parameters was carried out with assay kit supplied by Randox Industries Ltd. UK.

### Plant Material

The root of *R.longipedicellata* was collected by Mr. Felix Isang of Forestry Research Institute (FRIN) Ibadan. Mr.TK Odewo of the same Institute authenticated the plant. Voucher specimen of the plant with No FHI 106997 was deposited at the FRIN Herbarium.

### Extraction

100g of air-dried crushed plant material (root) were extracted with 500ml MeOH-H<sub>2</sub>O (8:2 v/v) at room temperature under reflux for 8 hours (by Soxhlet method). The extract solution was filtered, concentrated by air-drying on the bench and freeze-dried.

### Phytochemical Analysis

Phytochemical analysis of *R.longipedicellata* was performed according to the method of Sofowora<sup>7</sup>. The presence of alkaloids, glycosides, reducing sugars, tannins, saponins and flavonoids was determined.

### Animal Source

50 adult Swiss albino mice weighing between 20–25gm were purchased from the animal center of Federal Vaccine Production Laboratories, Yaba, Lagos. They were sorted into 5 groups of 5 mice each, housed in standard mouse cages, fed with livestock feed and water *ad libitum*.

### Acute Toxicity

Determination of the dose of root extract of *R. longipedicellata* that would kill 50% of the animal population (LD<sub>50</sub>) was undertaken according to the method of Litchfield and Wilcox as described by Aji *et al.*<sup>8</sup>.

Twenty-five male mice sorted into five groups of 5 mice each were used for the experiment.

They were fasted for 12 hours but were allowed water freely. Doses of 20g/kg, 15g/kg 10g/kg, 5g/kg and 2.5g/kg was administered orally with the aid of oral cannular and the animals were observed for 24hrs. Mortality in each group was recorded; the LD50 was calculated using the plot of percentage mortality against the log of dose.

### Tolerance Test

20 mice divided into groups of 10 mice each were used for this test. A group (test group) was administered the extract at a dose of 1500mg/kg for 30 days. The animals were bled on the 31<sup>st</sup> day and serum Alkaline phosphatase, Alanine Transaminase, Aspartate Transaminase were assayed. PCV, creatinine, bilirubin levels were determined.

### Parasites Source and Animal Infection

The parasites, *Plasmodium berghei* Keyberg174N strain were obtained from Nigerian Medical Research Institute (NIMR), Yaba- Lagos. 1ml of blood from a donor mouse (at peak parasitemia) was diluted with 4ml of phosphate buffer. The acclimatized mice were infected (sterile technique employed) intraperitoneally with 0.2ml of the diluted parasitised blood. Parasite density/ $\mu$ l of blood was estimated with the formula Parasites Count/WBC Count x 8000.

### Preparation of Extracts and Drugs for Bioassay

The plant extract was given to the test animals after suspension in a mixture of distilled water and 0.5% sodium carboxymethyl cellulose (CMC). Chloroquine and Artesunate in 0.5% CMC were used as reference drugs (positive control). The untreated animals (negative control) were experimentally handled like the test animals except that they received appropriate volumes of the dosing vehicle.

### Suppressive Activity

This was carried out using the method described by Datta<sup>9</sup>. Five groups of five mice each were infected with the *P. berghei* and 2 hours later were orally administered doses of 50 and 100mg/kg of the extract, chloroquine 8mg/kg and Artesunate 120mg/kg per day for 4 days respectively. A group was left untreated but was given same volume of the dosing vehicle (CMC).

Examination of the blood of each mouse for presence or absence of parasites started on the fifth day after the infection and administration of extracts and drugs. This continued every other day for 28 days or until death of animals.

### Therapeutic or Curative Activity

Using the same method described by Datta<sup>9</sup>, five groups of five mice each were allowed to express 2% parasitemia after 5 days of infection, and were orally administered the extract at doses of 50 and 100mg/kg, chloroquine 8mg/kg and Artesunate 120mg/kg per day for 4 days. A group was left untreated but was given same volume of the dosing vehicle (CMC). Examination of the blood of each mouse for presence or absence of parasites started on the ninth day after the infection and administration of extracts and drugs. This continued every other day for 28 days or until death of animals. Average percentage inhibition for suppressive and percent clearance rate for therapeutic activities for extract, chloroquine and artesunate treated mice were determined relative to the untreated mice (negative control).

## RESULTS

Phytochemical analysis of methanolic root extract of *R. longipedicellata* showed that it consisted of alkaloids, cardiac glycosides of cardinolides group, anthraquinone glycosides, pheloba tannins, saponins and flavonoids. Percentage yield of crude drug was 17% while LD<sub>50</sub> of the extract is 3650mg/kg, which gave a therapeutic index of 73 and 36.5 for 50mg and 100mg/kg doses. The results from biochemical analysis of the serum of the mice after administration of extract are summarized in Table 1.

Table 1: Biochemical analysis of blood after administration of *R. longipedicellata* extract

| Parameters                     | Control           | <i>R. longipedicillata</i> |
|--------------------------------|-------------------|----------------------------|
| PCV%                           | 36.50 $\pm$ 0.28  | 38.83 $\pm$ 0.16*          |
| ALP (UI/L)                     | 37.23 $\pm$ 0.37  | 34.59 $\pm$ 0.50*          |
| SGOT (UI/L)                    | 14.43 $\pm$ 0.21  | 12.28 $\pm$ 0.02*          |
| SGPT (UI/L)                    | 20.50 $\pm$ 0.84  | 15.64 $\pm$ 0.76*          |
| Creatinine ( $\mu$ mol/l)      | 187.06 $\pm$ 1.15 | 162.30 $\pm$ 0.15*         |
| Total Bilirubin ( $\mu$ mol/l) | 27.80 $\pm$ 0.23  | 26.46 $\pm$ 0.44           |

\* Significant

The antiplasmodial activity of the plant extract for suppressive and therapeutic activities are summarised in Tables 2-4.

**Table 2:** Average percent *parasitemia* for untreated, extract and reference drugs treated mice over a 28-day period.

| Treatment                          | Dose mg/kg | Parasite density per microlitre of blood |                  |
|------------------------------------|------------|--|------------------|
|                                    |            | Chemo-suppressive                        | Chemotherapeutic |
| Untreated                          | --         | 10729.94 ± 85                            | 14162.5 ± 50     |
| <i>R. longipedicellata</i> Extract | 50         | 764.04 ± 68                              | 7526.5 ± 50      |
|                                    | 100        | 5927 ± 75                                | 5447 ± 121       |
| Chloroquine treated                | 8          | 0  | 749.90 ± 91      |
| Artesunate treated                 | 120        | 340 ± 75                                 | 2947.91 ± 81     |

**Table 3:** Suppressive activity: percent inhibition, survival rate and survival period.

| Compounds          | Untreated | CQ  | Artesunate | <i>R. longipedicellata</i> |       |
|--------------------|-----------|-----|------------|----------------------------|-------|
| Dose (mg/kg)       | -         | 8   | 120        | 50                         | 100   |
| Clearance rate (%) | 0         | 100 | 100        | 86.8                       | 65.43 |
| Survival rate (%)  | 0         | 100 | 100        | 40                         | 0     |
| Survival period    | 23        | >28 | > 28       | >28                        | 19    |

**Table 4:** Chemotherapeutic: percent inhibition, survival rate and survival period

| Compounds          | Untreated | CQ    | Artesunate | <i>R. longipedicellata</i> |       |
|--------------------|-----------|-------|------------|----------------------------|-------|
| Dose (mg/kg)       | -         | 8     | 120        | 50                         | 100   |
| Clearance rate (%) | 0         | 80.85 | 100        | 36.73                      | 64.60 |
| Survival rate (%)  | 0         | 40    | 100        | 0                          | 0     |
| Survival period    | 23        | >28   | > 28       | 22                         | 25    |

## DISCUSSION

This study was designed to establish the tolerance and antimalarial activity of methanolic root extract of *R. longipedicellata*, which would justify its use as a tonic and in the treatment of malaria by traditional medicine practitioners. The high therapeutic indices of the extract point to a good safety margin for the plant extract. This substantiates the claim that herbal remedy might be safer than synthetic drugs<sup>5,6</sup>.

Results Results from biochemical analysis of serum ALP, ALT, AST, Bilirubin and Creatinine that presented a significant decrease might mean that there was no cellular injury causing enzyme leakage from the liver into the bloodstream. This suggests that the extract might have hepatic effect without producing cellular necrosis.

The observed increase in PCV (compared to control) coupled with the unchanged level of

bilirubin is suggestive of a positive haemopoetic effect of *R. longipedicellata*, justifying its use as a tonic in folk medicine. The root extract of *R. longipedicellata* exhibited antiplasmodial activity comparable to that of conventional drugs (chloroquine and artesunate) presently in use for the treatment of malaria (Tables 2-4). Results of the suppressive activity (2hrs post-infection) corroborate its use to prevent malaria in ethnomedicine. Its therapeutic use was also substantiated as it exhibited a good clearance rate (Table 4) at 100mg/kg (64.60%).

The extract activity for both preventive and curative was quite comparable to those of the reference drugs – artesunate and chloroquine. The suppressive activity of the extract was not dose dependent; a higher dose corresponded to a higher parasitemia (Table 2) which confirms the variation in drug sensitivity profile of each life cycle stage of the plasmodium<sup>10</sup>. However, the chemotherapeutic activity was dose dependent, which means a higher dose, is required to clear the parasites from the blood stream once

infection has been established. Further efforts to isolate and characterize the active component in this plant extract are going on.

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