

***In vivo* Antimalarial Activities of *Russelia Equisetiformis* in *Plasmodium Berghei* Infected Mice**

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The rising problem of resistance to most commonly used antimalarials remains a major challenge in the control of malaria suggesting the need for new antimalarial agents. This work explores the antiplasmodial potential of ethanol extract of *Russelia equisetiformis* in chloroquine *Plasmodium berghei* infected mice. Swiss albino mice were intraperitoneally infected with chloroquine-resistant *P. berghei* (ANKA). Experimental mice were treated for four days consecutively with graded doses of plant extracts and standard antimalarial drugs (artesunate and chloroquine) at a dose of 10 mg/kg body weight used as control. The extract showed a dose-dependent activity in the chemosuppression of *P. berghei* parasites by 31.6, 44.7, 48.4 and 86.5% at doses of 100, 200, 400 and 800 mg/kg, while chloroquine (10 mg/kg) and artesunate produced 59.4 and 68.4%, respectively. The extract showed a significant decrease in parasitaemia ($P < 0.05$). The level of parasitemia and decrease in weight in all the treated groups was significantly lower ($P < 0.05$) compared with the infected but untreated mice. The plant extract was devoid of toxicity at the highest dose tested (5000 mg/kg). The study concluded that the ethanol extract of *R. equisetiformis* possesses antimalarial effect, which supports the folk medicine claim of its use in the treatment of malaria.

Key words: Antiplasmodial, plant extract, *Russelia equisetiformis*, *Plasmodium berghei*

Malaria is still among the most important parasitic diseases worldwide as up to three million deaths due to malaria are recorded around the world annually, with Africa bearing more than 90% of the burden^[1]. In 2009, approximately 781 000 deaths among which 89% cases were found in the African region

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Accepted 08 August 2015

Revised 26 January 2015

Received 18 May 2014

Indian J Pharm Sci 2015;77(4):504-510

resulted from malaria^[2]. This parasitic disease is directly responsible for one in five childhood deaths in Africa and directly contributes to illness and deaths from diarrheal disease, respiratory infections and malnutrition^[3]. One of the reasons for continual increase in the global prevalence of malaria resulted from increasing resistance of the parasite to antimalarial drugs and this makes the search for new antimalarial drugs imperative^[4].

There is an urgent need for the discovery and the development of new effective and safe drugs. Over the years, plants have been important sources of new drugs and several medicinal plants continue to provide easily accessible alternatives to widely used antimalarials^[5]. It is also well known that most widely used curative antimalarial drugs such as quinine, obtained from cinchona species and artemisinins obtained from artemisia species are plant products^[6,7]. Also, plants remain the main source of many phytochemical compounds with antiplasmodial activity^[8].

Russelia equisetiformis of the family *Scrophulariaceae* is a medicinal plant used by traditional healers to treat malaria, cancer and inflammatory diseases and it is also claimed to promote hair growth among the Yoruba tribe in Nigeria^[9]. Personal communication with this tribe revealed that, the whole plant is used for the treatment of pain and inflammation^[10]. Medicinally, the plant is used for the treatment of diabetes and leukemia in Southwestern, Nigeria^[10]. The extract of the plant has been reported to possess antibacterial, analgesic and antiinflammatory activities^[11]. Recently, two flavonoid compounds isolated from *R. equisetiformis* were reported to have potential analgesic activity^[12]. Flavonoids, triterpenes saponins are known to either produce inhibitory, and/or stimulatory effects on convulsions^[13,14]. In addition to its antiinflammatory activity, *R. equisetiformis* has also been reported to have membrane stabilizing properties of which phenyl ethanoid glycosides of this plant, russectinol and russeliaoside, were identified as its active constituents^[11,15].

The molecular formula of russectinol was determined as $C_{29}H_{36}O_{15}$ by EI-MS^[12]. This EI-MS (negative-ion mode) of russectinol displayed a pseudo-molecular ion peak $[M-H]^-$ at m/z 623. IR ν_{max} KBr (cm^{-1}): 3424, 2937, 1825, 1594, 1363 and ¹H-NMR spectrum exhibited the presence of eight olefinic protons,

one methyl doublet signals, two anomeric proton signals (δ_{H1} 4.39 and δ_{H1} 5.20) confirmed by two anomeric carbons (δ_{C1} 103.20 and δ_{C1} 102.03)^[12]. The molecular formula of russeliaoside was determined as $C_{23}H_{26}O_{10}$ by EI-MS (negative-ion mode) of russeliaoside displayed a pseudo-molecular ion peak $[M-H]^-$ at m/z 461^[12]. The ¹H-NMR spectrum exhibited the presence of eight olefinic protons, one methyl doublet signals, one anomeric proton signal (δ_{H1} 5.21) confirmed by one anomeric carbon (δ_{C1} 102.07) indicating that the compound contains one sugar moiety (rhamnose)^[12].

Treatment of malaria by traditional methods 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^[16]. The traditional medicine of this continent constitutes an important source for ethno pharmacological investigations. In this paper, we report for the first time the *in vivo* antiplasmodial activities of *R. equisetiformis* using the suppressive and prophylactic model.

Sample of chloroquine resistant *P. berghei* ANKA clone, was received from Malaria Research Laboratory, Institute for Medical Research and Training (IMRAT), University College Hospital Ibadan, Nigeria and was used for the study to evaluate the antimalarial activity of the plant material used in this study. Each mouse was infected intravenously in the tail vein with 1×10^7 parasitized erythrocytes from an infected donor mouse. The day of infection was defined as day zero (D0) and subsequent days D1, D2, D3, D4, D5.

The plant sample was collected in the month of October 2012 from Bodija in the South West of Nigeria. The plant was identified in the herbarium of the Forest Research Institute, Ibadan, Nigeria, where voucher specimen was deposited with voucher number 106998. The whole plant excluding the root was air dried at room temperature and powdered in a grinding machine and 300 g of the powdered plant was macerated with 3 l of 90% ethanol (1:10 w/v) for 48 h and then filtered. The filtrate was dried with a rotatory evaporator. The residue was collected and stored for onward use. Standard chloroquine sulphate (Batch No. 442; May and Baker Nigeria Plc., Lagos) and artesunate (Mekophar Chemical

Pharmaceutical Joint-Stock Company, Vietnam) were used as standard reference for the antimalarial screening in this study.

The assessment of minimum lethal dose was carried out in two phases as previously described^[17]. In the phase 1 study, 3 groups of 3 mice each were used. The mice in the 3 groups were orally administered with doses of 10, 100 and 1000 mg/kg of the extract and monitored for 24 h for mortality. In the phase 2 study also 3 groups of 3 mice in each were used. The mice in the 3 groups were orally administered with 1600, 2900 and 5000 mg/kg of the extract and monitored for 24 h for mortality if any. The phase 2 study was carried out based on the result of the observation of mortality in phase 1 study. The lethal dose and the penultimate dose to the lethal dose would indicate the value of the LD₅₀^[18].

Swiss albino mice (18-25 g) obtained from the animal house of Biomedical Sciences, College of Health Sciences LAUTECH Osogbo, were acclimatized for 7 days before commencing the experiment. The mice were infected with 0.2 ml blood containing about 1×10^7 dose of *P. berghei berghei* (16.60%) from a donor mouse. Each mouse was inoculated on D0, (intraperitoneally) for the suppressive model and on the fifth day (D4) for the prophylactic model.

Peters' 4-day suppressive test against chloroquine-resistant *P. berghei* infection in mice was used for the evaluation of schizontocidal activity on early infection^[19,20]. The mice were divided into 7 groups of five mice each. The first 4 groups were administered 100, 200, 400 and 800 mg/kg/day doses of the extract for 4 consecutive days, while the groups 5 and 6 were administered chloroquine and artesunate 10 mg/kg/day and the group 7 was administered an equivalent volume (0.2 ml) of normal saline (control group) for 4 consecutive days (D1-D4) using oral cannula. On the fifth day (D5), thin blood films were prepared from blood collected from the tail and stained with 10% Giemsa and viewed under the X100 objective (oil immersion)^[21]. The numbers of infected erythrocytes were counted until 100 erythrocytes were achieved. The mean per cent suppression of parasitaemia was calculated in comparison to controls as, % suppression = $\frac{(\% \text{ parasitemia in negative control} - \% \text{ parasitemia in test group})}{(\% \text{ parasitemia in negative control})} \times 100$.

The prophylactic activity of the extract was performed as previously described^[20]. In this procedure, the adult mice were randomized into 7 groups of 5 mice each. Group 1 was treated with normal saline, groups 2 to 5 were treated with 100, 200, 400 and 800 mg/kg of the extract orally, group 6 and 7 (positive control) were treated with 10 mg/kg of the standard drug chloroquine and artesunate. Treatment was initiated on D0 and continued till day 4 when the mice were all infected (intraperitoneally) with the parasite. Blood smears were then made from each mouse 72 h after inoculation on day 7. The rectal temperature of each mouse was also determined daily by using a thermprobe thermometer. Blood samples were taken from the tail vein of each mouse for thin film microscopy and the number of the parasitized cells was determined and the percentage suppression evaluated^[22]. The body weight of each mouse in all the groups was taken before infection (day 0) and then daily up to day 5.

Experimental procedures and protocols used in this study conform to the "Guide to the care and use of animals in research and teaching" (NIH publications number 85-93 revised in 1985). One-way analysis of variance (ANOVA), followed by Student Newman-Keuls' test was used to analyse and compare results at a 95% confidence interval. Statistical significance was set at $P < 0.05$. The results were expressed as mean \pm SEM (standard error of mean).

The weight of the brown crude extract obtained from the extraction procedure was 11.10 g and the percentage yield was 4.6%. For the acute toxicity test, all the animals were still alive at the end of the experiment and the LD₅₀ value was greater than 5 g/kg and is of no harm. Hence, the extract can be declared harmless^[18].

In the suppressive test, the mean parasitaemia on day 5 post infection were 16.7 ± 1.5 , 13.5 ± 3.1 , 12.6 ± 0.6 and 3.3 ± 0.6 for 100, 200, 400 and 800 mg/kg, respectively. The value for control was 24.4 ± 3.7 while chloroquine and artesunate were 9.9 ± 0.9 and 7.7 ± 1 ($P < 0.05$). The percentage chemosuppression of the extract were 31.6, 44.7, 48.4 and 86.5%, respectively while those of chloroquine and artesunate were 59.4 and 68.4%. The % parasitaemia was observed to be decreasing with increasing dose levels of the extract, an indication that the highest dose exhibited the optimal chemo-suppression. These values

were significant in comparison to control ($P<0.05$) while 800 mg/kg, chloroquine and artesunate were highly significant to control ($P<0.05$). Similarly chloroquine and artesunate exhibited significant increase in % chemosuppression compared to control ($P<0.05$). While the percentage parasitaemia in chloroquine and artesunate were much lower than in extract at the lowest dose (fig. 1), the percentage parasitemia of the highest dose of the extract was lower in comparison to chloroquine and artesunate but the difference was not statistically significant. The % chemosuppression observed in chloroquine and artesunate was slightly different but not statistically significant ($P>0.05$, fig. 1). Also it was observed that the weight of the animals decreased 24 h post infection, which was reversed on 72 h (day 3) for the treated animals. The untreated animal group experience further decrease in weight after 72 h (Table 1).

Percent parasitaemia for 100, 200, 400 and 800 mg/kg were 14.14 ± 1.81 , 9.2 ± 0.40 , 8.08 ± 1.21 and 5.2 ± 0.4 , respectively. The value for control was 15.98 ± 0.83 while the values for chloroquine and artesunate were 7.5 ± 1.5 and 4.8 ± 0.6 , respectively. The % chemosuppression of the extract at 100, 200, 400 and 800 mg/kg were 11.51, 42.41, 49.44 and 67.46, respectively while that of chloroquine and artesunate were 53.07 and 70.0%. Similar to what was observed in the suppressive model, the % parasitaemia was observed to be increasing with increase in dose levels of the extract, indicating that optimal chemosuppression was also exhibited at the highest dose in this model. These values were highly significant in comparison to control ($P<0.05$). As observed in the suppressive model, chloroquine and Artesunate also exhibited a significant ($P<0.05$) decrease in % parasitaemia when compared to control. However, the % parasitaemia seen in chloroquine and Artesunate were significantly ($P<0.05$) lower than in extract at all dose levels except for the highest dose with chloroquine (fig. 2).

Fig. 3 summarises the results of the suppressive study of *Russelia equisetiformis* for the antipyretic investigations in malaria-induced mice. The temperature pattern from D0 to D5 showed that the decrease of temperature was prominent with extract (800, 400 and 100 mg/kg), chloroquine as compared to the control on day 1 which were all significant at day 2 as evidence of establishment

of malaria. However, the temperatures of mice that received extract (100, 400 and 800 mg/kg) were

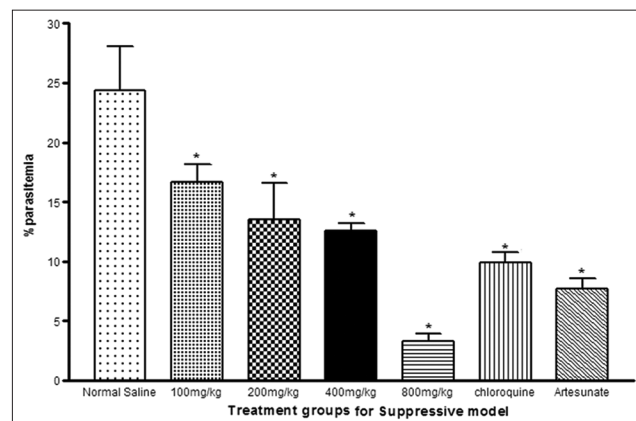


Fig. 1: Suppressive effect of ethanol extract of *Russelia equisetiformis* on chloroquine resistant *Plasmodium berghei* in mice. The bars are expressed as mean±SEM, (n=5), *indicates $P<0.05$ when compared with normal saline.

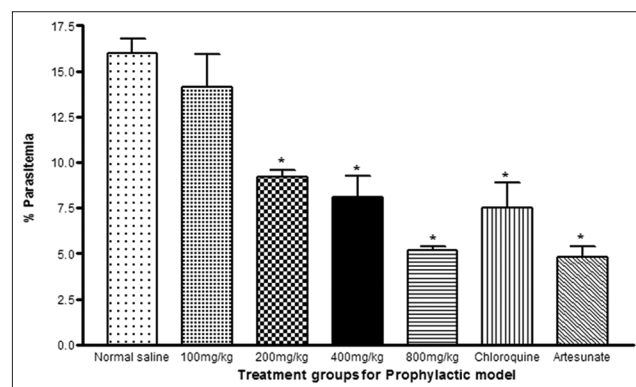


Fig. 2: Prophylactic effect of ethanol extract of *Russelia equisetiformis* on chloroquine-resistant *Plasmodium berghei* in mice. The bars are expressed as mean±SEM, (n=5), *indicates $P<0.05$ when compared with normal saline.

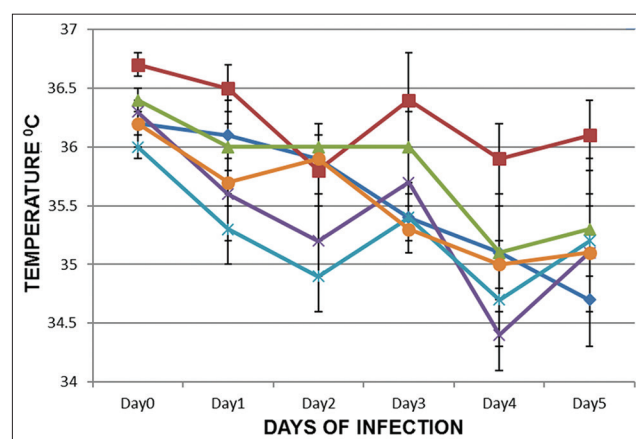


Fig. 3: The antipyretic activity of ethanol extract of *Russelia equisetiformis* in malaria induced mice for suppressive model. The lines with bars are expressed as mean±SEM, (n=5). Control (◆); 100 mg/kg (■); 200 mg/kg (▲); 400 mg/kg (×) and 800 mg/kg (✱).

TABLE 1: EFFECTS OF MALARIA ON BODYWEIGHT

Dose mg/kg	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Control	26.6±0.7	25.6±0.4	25.4±0.9	25.3±1.0	25.6±0.7	25.4±0.9
100 mg/kg <i>R. equisetiformis</i>	25.2±1.4	23.7±1.4	25.2±1.4	24.8±1.5	25.5±1.5	25.1±1.4
200 mg/kg <i>R. equisetiformis</i>	25.4±1.5	24.3±1.3	23.5±1.5	23.7±1.8	24.7±2.1	24.3±2.5
400 mg/kg <i>R. equisetiformis</i>	19.8±1.0	19.7±1.1	20.0±1.0	20.0±1.1	20.7±1.1	20.7±1.1
800 mg/kg <i>R. equisetiformis</i>	22.8±0.9	21.5±0.9	22.3±0.6	22.2±0.7	22.3±0.8	21.8±0.8
Chloroquine	26.5±0.2	25.7±0.2	26.1±0.3	26.1±0.2	26.1±0.2	26.4±0.3

Values are expressed as mean±SEM, (n=5). SEM: Standard error of mean, *R. equisetiformis*: *russelia equisetiformis*

increased compared to that of control. The reversal of temperature, which was prominent with 100 mg/kg is contrary to an optimal activity. Similar pattern was observed in the prophylactic model (result not shown).

The present study investigates the antiplasmodial activities of ethanol extracts of *R. equisetiformis* for the treatment of malaria infection using the 4-day suppressive test and prophylactic model. The 4-day suppressive test commonly used for antimalarial screening and the determination of percent inhibition of parasitemia is the most reliable parameter^[19]. A mean group parasitemia level of less than or equal to 90% of mock-treated control animals usually indicates that the test compound is active in standard screening^[23]. The *in vivo* results of the extracts at different doses show that they are capable of reducing the level of parasites in circulation both in suppressive and prophylactic models. Their activities were observed to be dose dependent and significantly higher amounts of the crude extracts were required to elicit such activities when compared to artesunate and chloroquine used as standard drugs in the study. This significant suppression of parasitemia by the ethanol extract of *R. equisetiformis* in divided doses on day 4 is in agreement with previous studies with ethanol extract of *Asperagus africanus*^[24] and *Azadirachta indica*^[25]. The phytosteroids and flavonoids detected in *R. equisetiformis* could as well be responsible for the antimalarial effect as these metabolites have been proved to possess potential immunomodulatory effects in other plants^[26]. In view of this, the extract can be considered to contain some antimalarial active ingredients that could serve as a template for the production of relatively inexpensive antimalarial drugs. It is clear from our results that the percentage parasitemia of *P. berghei* infected mice treated with extract of *R. equisetiformis*, changed significantly ($P \leq 0.05$) in comparison to the non-treated infected mice. Moreover, the ethanol extract administered at a dose of 800 mg/kg per

day for five days resulted in >50% reduction in percent parasitemia, a performance that may likely be improved upon if the crude extract is purified to identify and isolate active substituents. The increase in activity observed in the extract as the dose increases in both the suppressive and prophylactic studies explains the fact that the crude extract exerted a pronounced activity against the malaria parasite at an optimal dose of 800 mg/kg.

The oral median lethal dose (LD_{50}) of the crude extract was estimated to be ≥ 5 g/kg orally since there were no mortality at all dose levels used^[17]. The absence of death following the oral administration of *R. equisetiformis* extract at 5000 mg/kg observed in mice implies that the extract is practically non-toxic. It has previously been noted that any chemical that exhibited an LD_{50} more than 5000 mg/kg is practically non-toxic based on Hodge and Sterner Toxicity Scale^[27]. On the contrary, it was shown that the methanol extract of the plant has an oral LD_{50} of 3.6 g/kg when administered intraperitoneally^[28]. The oral administration has been reported to be about 100 times less toxic than intraperitoneally^[29]. Also, it has been previously noted that that acute toxicity test on the same extract or drug yielded different values from laboratory to laboratory^[17]. Acute toxicity tests with the extracts have also demonstrated their safety because the highest dose used for the screening did not cause death. Interestingly, the highest dose used to treat parasite-infected mice, which elicited antimalarial activity, was much lower than the highest acute dose.

There has been no previous report on the antipyretic activity of the ethanol extract of the plant and this has prompted the evaluation of the plant for its antipyretic activity. Malaria is known to induce high body temperature in humans^[30], and low temperature in rodent mice^[31]. Using the two models of malaria treatment, it was shown that the extract displayed antipyretic activity at all the models by raising the

body temperature in the malaria-infected mice. The reversed temperature displayed was optimum at 100 and 800 mg/kg in suppressive and prophylactic model respectively when compared to the control. This activity is on contrary with the optimum antimalarial activity by the same dose in malaria suppressive model, but correlates with optimum activity by the same dose in malaria prophylactic model, seeing that the extract remarkably reversed the body temperature that was lowered by malaria parasites in this study. In ethnomedicine, other works have shown that *Dodonaea angustifolia* seed and *Azadirachta indica* leaves as antimalarial agents were able to reverse the temperature induced by malaria parasites^[32,33]. This comparison explains the fact that traditional plants are good remedies for malaria infection and fever as claimed in the folklore.

Our results suggest that the ethanol extract of *R. equisetiformis* show some intrinsic antimalaria activity by its percentage chemo suppression and prophylactic ability against chloroquine-resistant *P. berghei* parasites. This performance can surely be improved upon in future studies if the crude extract is purified and the active substituents identified. The extracts have considerably low or no toxicities in experimental mice. This findings support the traditional use of this plant for the treatment of malaria.

Acknowledgements:

The authors are grateful to Dr. G. O. Gbotosho, Malaria Research Laboratory, Institute for Medical Research and Training (IMRAT), University College Hospital Ibadan, Nigeria for providing the malaria parasite used for the study, also to Mrs Sakirat Braimah for technical assistance.

Financial support and sponsorship:

Nil.

Conflicts of interest:

There are no conflicts of interest.

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