

Prophylactic, Suppressive And Curative Antiplasmodial Potentials Of Methanol Root Extract Of *Napoleona imperialis* In *Plasmodium berghei berghei* Infected Male Albino Mice

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Abstract : The prophylactic, suppressive and curative anti-plasmodial potentials of methanol root bark extract of *Napoleona imperialis* in *Plasmodium berghei berghei* infected male albino mice were investigated. Sixty-five (65) albino mice weighing between 15 – 22g were used for the study. They were divided into 13 groups with 5 mice in each group. Group 1 served as control and was parasitized but not treated. Groups 2, 3, 4 and 5 served as prophylactic model and were treated daily with 200, 400, 600 mg/kg of *N. imperialis* root extract and 1.2mg/kg of pyrimethamine respectively for 3 days followed with the parasite being inoculated in day 4. Groups 6, 7, 8 and 9 served as suppressive model and were inoculated with the parasite with simultaneous daily treatment with 200 mg/kg, 400 mg/kg, 600 mg/kg of *N. imperialis* root extract and 5 mg/kg of chloroquine respectively for 3 days. Groups 10, 11, 12 and 13 served as curative model and were inoculated with the parasite followed by daily treatment with 200 mg/kg, 400 mg/kg, 600 mg/kg of *N. imperialis* root extract and 5 mg/kg of chloroquine respectively for three days. At the end of treatment, blood sample obtained from each mouse was examined under light microscope to reveal parasitized erythrocytes. The prophylactic model showed parasite suppression of 99.10%, 74.75%, 85.84% and 67.85% for 200, 400, 600mg/kg of *N. imperialis* and 1.2mg/kg of pyrimethamine respectively. The suppressive model showed a percentage parasite suppression of 99.10, 96.88, 99.67 and 99.71 for 200, 400, 600 mg/kg of *N. imperialis* and 5.0mg/kg of chloroquine respectively. In the curative model, parasitaemia clearance was only significant in the group treated with 200mg/kg of *N. imperialis* root bark extract. The study has revealed that the methanol root extracts of *N. imperialis* have good antimalarial prophylactic and suppressive activity on *Plasmodium berghei*-infected albino mice.

Keywords: *Napoleona imperialis*, *Plasmodium berghei berghei*, Malaria, Antiplasmodial Activity

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I. Introduction

Malaria is a very important parasitic infectious disease of humans [1]. Chloroquine has been the most widely used anti-malaria, however, the spread of resistance to the drug led to its withdrawal from use in most countries in sub-Saharan Africa in the 1990s [2]. The resistance of malaria parasite to Chloroquine and other anti-malaria has driven scientists into an intensive search for more effective agents against the scourge. Artemisinin combination therapies (ACTs) are currently the drug of choice in the treatment of malaria. However, there are reports of parasite resistance to the newly developed artemisinin in some regions [3]. Thus, there is an urgent need for increased efforts in anti-malarial drug discovery especially in Africa [4]. In recent times, natural products of plant sources have been the center of focus as the main source of new, safer and more effective bioactive compounds with medicinal properties [5]. An appreciable level of studies has been done on African traditional medicinal plants, ranging from ethno botanical surveys, to the actual extraction of the active ingredients in the plants [6,7]. It has been noted that in traditional practice, several plants are often used in combination [8]. One of such plants is the *Napoleona imperialis* which is a wild plant found in South Eastern Nigeria. *N. imperialis* is an evergreen non-timber plant that grows abundantly in bush fallows, secondary bushes and marginal lands in most of the tropical humid zones of West Africa [9]. The plant belongs to the family known as the lecythidaceae, along with the cannon ball tree (*Corrupita guianensis*), which grows in most regions of Nigeria [10,11]. The plant is commonly known as “Utum” in the Ikwano dialect and “Mkpodu” in Igbo language of Nigeria [12]. The chemical composition of the leaf, bark and roots of *Napoleona imperialis* have been documented [13,9]. The leaves and root extract of the plant have been reported to contain glycosides, tannins, proteins, flavonoids and saponins [14,15]. The leaves have a characteristic bitter taste, foaming properties, and can cause injuries to the digestive mucosa and hemolytic changes in blood [16].

Study revealed that the plant extract has a better wound healing property (100% efficacy) than a standard antibiotic, Cicatrin. [17]. It has been reported that oral administration of methanol extract of *N. imperialis* may be useful for prevention and treatment of hypertension [14]. Evaluation of antimicrobial properties of ethyl acetate extract of the root of *N. imperialis* showed the extract had activities against certain bacteria confirming the use of the plant in ethno-pharmacology, [18]. The anti-plasmodial potential of the leaves of *N. imperialis* have been evaluated to justify its usage in traditional medicine for treatment of malaria [19]. The present study is therefore aimed at investigating the anti-malarial potentials of Methanol root extract of *N. imperialis* in mice.

II. Materials And Methods

2.1 Plant Materials

The plant *N. imperialis* was obtained from Mgbirichi community in Imo state and identified at the herbarium of University of Nigeria, Nsukka. The *N. imperialis* roots were washed clean, dried and pulverized into fine powder. The powdered root was macerated in 80% methanol and allowed to solubilize. The filtrate was obtained and concentrated in a Waterbath at 45⁰C. The crude extract was obtained and preserved in refrigerator at -4⁰C.

2.2 Malaria Parasite

A chloroquine – sensitive Strain of *Plasmodium berghei-berghei* was obtained from National Institute of Medical Research (NIMR) in Lagos, Nigeria and Maintained by sub-passage in mice. The parasitized blood donor with high parasitaemia was obtained by first anaesthetizing the mouse with chloroform, and through cardiac puncture, blood was collected using sterile syringe into heparinized bottles. The percentage parasitaemia was determined by counting the number of parasitized red blood cells against the total number of red blood cells. The desired volume of blood obtained from the donor mouse was suitably diluted with sterile normal saline so that the final inoculum (0.2 ml) for each mouse contained the required number of parasitized red blood cells (1.0 x 10⁷ parasitized red blood cell). The method described by Okokon *et al.*, [20] was employed in inoculating the parasite in the experimental animals.

2.3 Animals And Experimental Design

Sixty-five (65) inbred male albino mice (15 – 22 g) were obtained from the animal house of Pharmacology and Toxicology Department, University of Uyo. They were maintained at standard laboratory condition; twelve hours' light – dark cycle, fed with rat pellet feed with clean drinking water *ad libitum*. Parasitized red blood cells containing *Plasmodium berghei-berghei* was injected through intraperitoneal route into the mice. The mice were divided into thirteen groups with 5 animals in each group. Group 1 served as the control (received no treatment). Groups 2, 3, 4 and 5 served as prophylactic model and were treated daily with 200 mg/kg, 400 mg/kg, 600 mg/kg of *N. imperialis* root extract and 1.2 mg/kg of pyrimethamine respectively for 3 days followed with the parasite being inoculated in day 4. 72 hours (3 days) after the inoculation of the parasite, the animals were sacrificed and blood sample obtained for analysis. Treatment with pyrimethamine is the standard prophylactic antimalarial therapy and served as the positive control for comparison in the prophylactic model. Groups 6, 7, 8 and 9 served as suppressive model and were inoculated with the parasite with simultaneous daily treatment with 200 mg/kg, 400 mg/kg, 600 mg/kg of *N. imperialis* root extract and 5 mg/kg of chloroquine respectively for three days. Groups 10, 11, 12 and 13 served as curative model and were first inoculated with the parasite followed by daily treatment with 200 mg/kg, 400 mg/kg, 600 mg/kg of *N. imperialis* root extract and 5 mg/kg of chloroquine respectively for three days. The treatment started 72 hours (3 days) after the parasite were inoculated in the mice. Leishman's stained thin smears were prepared from the tail blood of each animal on Day 0, Day 1, Day 2 and Day 3 of treatment.

2.4 Evaluation of Antimalarial Activity

Thin blood films obtained from each mouse in all the group was examined under the light microscope to reveal parasitized erythrocytes in a random field of a microscope. The percentage parasitaemia was obtained by counting the number of parasitized blood cells out of 500 erythrocytes in random fields of microscope.

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

2.5 Statistical Analysis

Data obtained from this Study were analyzed using the Statistical package for Social science (SPSS) Version 20.0 for windows. Analysis of Variance (ANOVA) were used to compare means and values were considered significant at P < 0.05. Post hoc multiple comparisons for ANOVA were done using least significant difference (LSD).

III. Results

3.1 Prophylactic Antiplasmodial Effect of Methanol Root Extract of *N. imperialis* on *Plasmodium berghei* infected mice

The prophylactic antiplasmodial effect of methanol root extract of *N. imperialis* in albino mice infected with *Plasmodium berghei berghei* is presented in Table 1. The result shows that there was no significant difference ($p > 0.05$) in the *P. berghei* parasitaemia in the mice treated with different concentrations of *N. imperialis* methanol extract compared to the control and the chloroquine treated mice.

Table 1: Prophylactic Antiplasmodial Effect of Methanol Root Extract of *N. imperialis* on *Plasmodium berghei* infected mice

Treatment/Dosage	Parasitaemia	% Suppression
Group 1 – Control	10,678.00 ± 8,736.10	-
Group 2 – 200 mg/kg/day	96.67 ± 66.73	99.10
Group 3 – 400 mg/kg/day of <i>N. imperialis</i>	2,626.70 ± 1132.57	75.40
Group 4 – 600 mg/kg/day of <i>N. imperialis</i>	1,511.70 ± 1,494.20	85.84
Group 5 – 1.2 mg/kg/day of Pyrimethamine	3,433.30 ± 1,566.67	67.85

Values are presented as Mean ± SEM. * = Significant difference at $P < 0.05$ when compared to control.

Suppressive Antiplasmodial Effect of Methanol Root Extract of *N. imperialis* on *Plasmodium berghei* infected mice

The suppressive antiplasmodial model revealed that at 200, 400 and 600 mg/kg of *N. imperialis* methanol extract the *P. berghei* parasitaemia in the mice was significantly lower ($p < 0.05$) compared to the parasitaemia in the control. As shown in Table 2, at 600 mg/kg the extract was most effective in suppressing *P. berghei* parasitaemia in the mice with the parasitaemia not significantly ($p > 0.05$) different from the parasitaemia in the chloroquine treated mice.

Table 2: Suppressive Antiplasmodial Effect of Methanol Root Extract of *N. imperialis* on *Plasmodium berghei* infected mice

Treatment/Dosage	Parasitaemia	% Suppression
Group 1 – Control	10,500.00 ± 50.08	-
Group 6 – 200 mg/kg/day	105.00 ± 88.08*	99.10
Group 7 – 400 mg/kg/day of <i>N. imperialis</i>	328.33 ± 12.75*	96.88
Group 8 – 600 mg/kg/day of <i>N. imperialis</i>	35.00 ± 5.00*	99.67
Group 9 – 5 mg/kg/day of Chloroquine	30.00 ± 15.28*	99.71

Values are presented as Mean ± SEM. * = Significant difference at $P < 0.05$ when compared to control.

Curative Antiplasmodial Effect of Methanol Root Extract of *N. imperialis* on *Plasmodium berghei* infected mice

Curative effect of methanol root extract of *N. imperialis* on *Plasmodium berghei berghei* infected mice is presented in Table 3. The result shows that at day zero there was no significant difference ($P > 0.05$) in the *P. berghei* levels in the mice treated with *N. imperialis* root extract treated rats compared to the chloroquine treated and the control. After one day of observation the mice, *P. berghei* levels were significantly lower ($P < 0.05$) in the 600 mg/kg *N. imperialis* root extract and chloroquine treated rats compared to the control. On day two and three the *P. berghei* levels in the mice were higher in the control compared to the extract and chloroquine treated mice although the mean differences were not statistically significant ($P > 0.05$). Furthermore, parasitaemia clearance was significantly evident only in the 200mg/kg extract treated group.

Table 3: Curative Antiplasmodial Effect of Methanol Root Extract of *N. imperialis* on *Plasmodium berghei* infected mice

Treatment/ Dosage	Parasitaemia – Day 0	Parasitaemia – Day 1	Parasitaemia – Day 2	Parasitaemia – Day 3	% Clearance
Group 1 – Control	4,166.70 ± 927.96	19,500.00 ± 5,530.22	12,000.00 ± 2,753.97	6,516.70 ± 2,630.01	-
Group 10 – 200 mg/kg/day	10,500.00 ± 7,023.77	21,333.33 ± 2948.63	4,666.70 ± 927.69	2,006.70 ± 1,034.43	80.9
Group 11 – 400 mg/kg/day	12,333.00 ± 2,420.97	7,833.33 ± 3,876.57*	10,000.00 ± 3,329.16	10,833.0 ± 7,339.01	12.1
Group 12 – 600 mg/kg/day	5,031.70 ± 4,984.17	4000.00 ± 289.00*	8000.00 ± 2753.79	5,166.70 ± 1013.79	-
Group 13 – 5 mg/kg/day of Chloroquine	4,166.70 ± 2920.78	2833.00 ± 441.00*	7,677.00 ± 6,451.00	6,666.70 ± 2,455.15	-

Values are presented as Mean ± SEM. * = Significant difference at $P < 0.05$ when compared to control.

VI. Discussion

Malaria is a mosquito-borne infectious disease of humans and other animals caused by protozoan - a group of single celled microorganism belonging to the plasmodium genus [21]. Anemia, fever, nausea and flu-like illness are the common manifestations of malaria with coma and death reported in severe cases [22]. Development of drug resistance by plasmodium species and the resistance of anophelid mosquitoes to many insecticides have compounded the control of malaria in infested areas [23]. High cost of antimalarial drugs, its unavailability in certain areas, existence of resistance and reported toxicity of the available antimalarial drugs have warranted the inquiry into alternative sources of drugs for malaria treatment especially in the rural areas of developing and undeveloped countries of the world.

N. imperialis root is used locally for several purposes. The phytochemical screening of the root of *N. imperialis* has revealed the presence of alkaloids, tannins, saponins, resins, flavonoids, protein, carbohydrate and steroids [9]. Alkaloids, saponins, tannins and flavonoids have been reported to be responsible for antimalarial activities in plants [24]. The presence of these phytochemicals in methanol root extract of *N. imperialis* as reported by Etim *et al.*, [9] suggest that the plant has antimalarial activity. These secondary metabolites may have elicited the observed antimalarial activity either singly or in synergy with each other [25].

The prophylactic model of the study reveals that the root extract of *N. imperialis* had a significant chemoprophylactic activity when compared to the control. The high parasite count in the repository test may be attributed to the metabolism of the administered extract (before inoculation) to an inactive product. The report corroborates the study by Alli *et al.*, [26] who also reported high levels of parasitaemia clearance in the prophylactic study of the root extract of *Acacia nilotica*. Dose dependent significant chemosuppressive activity was observed in the suppressive model of the study when the extract treated groups were compared to the control. A compound is said to be active when its percentage chemosuppression in parasitaemia is 30 and above [27,28]. A plant with percentage suppression of 50 and above is categorised as good and very good [29]. The chemosuppressive activity of *N. imperialis* corroborates with similar studies on several other antiplasmodial medicinal plants such as *Dicliptera verticillata*, *Agelanthus dodoneifolius* and *Sida rhombifolia* [25,30,31].

In the curative model, the *N. imperialis* root extract at an extract concentration of 200 mg/kg showed gradual reduction in the parasitaemia level from day one to day three even more than the reference drug chloroquine. Chloroquine has a very high volume of distribution as it diffuses into the body adipose tissue [25]. The drug concentrates in the acidic food vacuoles of the parasite and interferes with the essential processes [32]. Chloroquine enters the red blood cells, inhibiting parasite cell and digestive vacuole by simple diffusion [33]. In this research result, chloroquine at day zero and day one reacted positively by reducing the malaria parasite level. But at day two and three, there was a development of resistance. The effectiveness of chloroquine against *Plasmodium falciparum* has declined as resistant strains of *Plasmodium falciparum* evolved.

The mechanism of action of the extract of *N. imperialis* has not been elucidated but some plants are known to exert antimalarial action by causing elevation of red blood cell oxidation or by inhibiting protein synthesis [34]. Phytochemicals in plants have been documented to be responsible for the antiplasmodial potentials of medicinal plants [35]. Alkaloids have been known to exhibit antimalarial properties by blocking protein synthesis in *Plasmodium falciparum* [36]. Flavonoids have been reported to chelate with nucleic acid base pairing of the parasites [37]. The extract could have elicited its action through either of the mechanisms mentioned above or by some other unknown mechanisms.

VII. Conclusion

From this study, it can be concluded that, the methanol root extracts of *N. imperialis* have shown a good antimalarial prophylactic and suppressive activity on *Plasmodium berghei*-infected albino mice.

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