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In vivo Antiplasmodial Activity and Haematological Parameters of the Methanolic Extract of *Clerodendrum polycephalum* Baker Leaves on *Plasmodium berghei berghei* in Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Authors COA and ABO designed the work. Author FBA contributed the laboratory work and drafted the paper. Author NOO supervised the antimalarial laboratory work while author EOI analyzed the data. All authors agreed to the review of the manuscript and agreed to submission.

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Original Research Article

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ABSTRACT

Aim: To evaluate the antimalarial effect of the methanol extract of the leaves of *Clerodendrum polycephalum.*
Study Design: *In-vivo* assay using albino mice. Three models were used: suppressive, prophylactic and curative.
Place and Duration of Study: The study was carried out at Drug Research and Production Unit, Faculty of Pharmacy, OAU, Ile-Ife, Nigeria from October 2013 to June 2014.
Methodology: Antimalarial activities were evaluated using methanolic extract of *Clerodendrum*

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polycephalum and mice containing 1.0×10^7 parasitized red blood cells. Suppressive, prophylactic and curative assays were done using 100 – 600 mg/kg body weight. Positive control mice received chloroquine 10 mg/kg in suppressive and curative assays while those of prophylactic received pyrimethamine 1.2 mg/kg body weight. In acute oral toxicity assay, three mice were sequentially dosed with 5000 mg/kg body weight at intervals of 48 hrs; observations were made on the nervous system. Sub-chronic oral toxicity of extract was assessed using Haematological parameters.

Results: At 100 – 600 mg/kg, prophylaxis and suppressive tests exhibited 57.46 – 91.56% chemosuppression and 57.86 -92.63% chemosuppression respectively. The curative test produced concentration dependent chemosuppression from day 3 – day 7; at 600 mg/kg, chemosuppression increased from 71.35 (day 3) to 86.37% (day 7). Positive controls significantly compared well with the activities of the extract (P=.05). By 10^{th} day in acute oral toxicity assay, slight thinning of the fur in the anterior area and loss of appetite were observed. At the end of the assay period, no mortality was observed. Treated animals in the sub-chronic oral toxicity test showed slight decrease and then increase in body weight. Furthermore, slight differences were observed in the values of the haematological parameters (red blood cell count, packed cell volume, haemoglobin estimation) compared to the control (P= .05).

Conclusion: The study corroborates the ethnomedicinal use of *Clerodendrum polycephalum* as a potent antimalarial remedy. Its use should be encouraged as an alternative to conventional antimalarial drug.

Keywords: Clerodendrum polycephalum; Plasmodium berghei berghei; suppressive; curative; prophylactic; toxicity.

1. INTRODUCTION

The genus *Clerodendrum* L. (family: *Lamiaceae*) is very widely distributed in tropical and subtropical regions of the world and is comprised of small trees, shrubs and herbs. More than five hundred species of the genus are identified till now and are widely distributed in Asia, Australia, Africa and America. Ethno-medical important of various species of *Clerodendrum* genus has been reported in various indigenous systems of medicines and as folk medicines. The genus is being used as medicines specifically in Indian, Chinese, Thai, Korean, Japanese systems of medicine for the treatment of various life threatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension. Few species of the genus like *Clerodendrum inerme, C. thomosonae, C. indicum*, and *C. speciosum* are ornamental and being cultivated for aesthetic purposes [1,2]. The powder/paste form and the various extracts of root, stem and leaves are reported to be used as medicine for the treatment of asthma, pyreticosis, cataract, malaria and diseases of blood, skin and lung. Anti-inflammatory, anti-oxidant, antimalarial, anti-microbial antihypertensive, antitumor, antidiabetic, antidiabetic, antihyperlipidemic, larvicidal and antidiarrhoel activities have been investigated and reported in literatures in order to proof their ethno-medical uses.

Little is known about the biological activities of *C. polycephalum* from literatures. The description of the plant has been given [3]. It is a climbing or erect shrub up to 15 ft high. The leaves and branches are yellow or brown pilose. The flowers are usually small, white, and arranged in head like clusters. The infusion of the leaves is used as antidote for snake poison [4]. Recently, it was reported that the leaves of *C. polycephalum* are rich in tannins, saponins, flavonoids, resins, balsams, alkaloid and phlobatannins [5]. The decoction of the leaves is also used as antimalarial in south western area of Nigeria. As part of our search for potent antimalarial natural products and the need to confirm the claim as antimalarial plant, this paper reports the investigation of antimalarial property of the methanolic extract of the leaf against *Plasmodium berghei berghei* in mice.

2. MATERIALS AND METHODS

2.1 Plant Collection, Identification and Extraction

The plant was collected at an abandoned plot in Ile-Ife, Nigeria (7° 29’ 40’’ N/ 4° 33’ 27’’ E) and taken to the herbarium unit of department of Botany, Obafemi Awolowo University, Ile-Ife for identification. Voucher specimen was deposited and a reference number IFE 16962 attached to it. Approximately 700 g of air dried leaf was soaked

in methanol and left in a shaker for 72 hrs. It was then filtered and concentrated to dryness and kept in the refrigerator until when needed.

2.2 Parasites Used

Plasmodium berghei berghei (NK 65) was obtained from the malaria centre (IMRAT) at the University College Hospital, Ibadan and maintained in the laboratory in Albino mice.

2.3 Ethics Statement

All mice used were kept in the laboratory and acclimitised for a week before studies began. All animal studies were conducted in accordance with "Principles of Laboratory Animal Care" guidelines and procedures [6] and approved by Ethics and Research Committee.

2.4 Parasites Inoculums Preparation

The parasitized blood from donor with about 20-32% parasitemia was got by first anaesthetising the mouse with chloroform, and through the cardiac puncture, using sterile disposable syringes. The number of parasitized red blood cells in a volume of blood cells was then calculated by multiplying the % parasitemia by the total number of red blood cells. The desired volume of blood then obtained from the donor mouse was suitably diluted with sterile normal saline so that the final inoculums (0.2 ml) for each mouse would contain the required number of parasitized red blood cells (that is 1.0×10^7 parasitized red blood cells).

2.5 Acute Toxicity (Assessment of Minimum Lethal Dose)

The method used has been described [7]. Three groups of 2 mice each were given doses of 1000, 3000 and 5000 mg/kg body weight respectively and monitored for 24 hrs, observing for mortality. The lethal dose and the penultimate dose to the lethal dose determined the LD_{50}.

2.6 Evaluation of the Suppressive Antimalarial Activity of *Clerodendrum polycephalum* (*Lamiaceae*) on *Plasmodium berghei berghei*

Suppressive test was done using the 4-day antimalarial chemosuppressive method [8]. Groups of *Plasmodium berghei berghei*-infected mice (5 per group) were treated with 100, 200, 400 and 600 mg/kg body weight respectively of the extract, one other group received chloroquine (10 mg/kg) as the positive control while another group received 0.9% normal saline as the negative control. All the test substances were administered orally once daily for four consecutive days (Day 0 to day 3). On day 4 (the fifth day), thin blood film was made from the tail of each mouse and the %parasitaemia and the average % chemo-suppression of parasitaemia in each group was assessed as shown below:

groups X 100/Average % parasitaemia in control group

That is: $\frac{\text{Control mean - Dose mean}}{\text{Control mean}} \times \frac{100}{1}$

The means were calculated as Mean ± Standard Error of Mean (SEM) where

$$\text{SEM} = \frac{\text{Standard deviation}}{\sqrt{n}}.$$

2.7 Evaluation of the Prophylactic Antimalarial Activity of *Clerodendrum polycephalum* (*Lamiaceae*) on *Plasmodium berghei berghei*

The test was performed by treating animals with extract before exposure to parasite [9]. Albino mice of either sex used for the prophylactic assay and were allowed to acclimatise with the laboratory environment for 2 weeks. The mice were divided into six groups of five in each. The first group was used as the negative control; the second group was treated with a dose of 100 mg/kg of the extract while groups 3, 4 and 5 were treated with doses of 200 mg/kg, 400 mg/kg and 600 mg/kg respectively. The last group was used as the positive control in which standard drug pyrimethamine 1.2 mg/kg was used. The treatment continued for 3 days before the inoculation of the plasmodium parasite. The tail smears of the animals were then taken 72 hrs later to assess the % parasitaemia.

2.8 Evaluation of the Curative Antimalarial Activity of *Clerodendrum polycephalum* (*Lamiaceae*) on *Plasmodium berghei berghei*

Antimalarial curative test was done by treating infected animals with plant extract [10]. Six groups of 5 mice each were used for the experiment. Four of the groups were treated with

the extract at the doses of 100, 200, 400 and 600 mg/kg body weight respectively. The fifth group received chloroquine (10 mg/kg) and the sixth 0.9% normal saline as the positive and negative control groups respectively. All the mice were first infected with *Plasmodium berghei berghei* and left for 72 hours for the infection to be established. On day 3, (infection day is D0), the different doses of the test agents were administered to the mice for 5 days. The parasitaemia were determined by taking the thin blood films of each mouse on days 3, 5 and 7, staining with giemsa stain and observing them.

2.9 Acute Oral Toxicity Studies

Acute oral toxicity and sub-chronic oral toxicity methods have been described accordingly [11]. Three mice were sequentially dosed with 5000 mg/kg body weight at intervals of 48 hrs. Once daily, observations such as changes in skin, fur, eyes, salivation, lacrimation, perspiration, (autonomic), drowsiness, gait, tremors and convulsions (central nervous system) were made. The assay was run for two weeks.

2.10 Sub-chronic Oral Toxicity

Three groups of 5 animals each were administered with distilled water and 500 mg/kg and 1000 mg/kg body weight of the extract respectively. The drug was administered daily for 28 days and the animals were observed for morbidity and mortality. Body weights of the animals were obtained weekly. On the 29th day, after an overnight fast, the animals were sacrificed and blood samples were collected in EDTA tubes. Haematological parameters (red blood cell count, packed cell volume, haemoglobin estimation) were then deduced from the samples.

Results of the three antimalarial models were analysed using Microsoft Excel statistical package while the haematological parameters were analysed using PRIMER.

3. RESULTS AND DISCUSSION

Clerodendrum species have been reported in ethnomedicine to be effective in the treatment of various ailments. The various parts (roots and leaves) were employed in the remedy of diseases ranging from fever, jaundice, typhoid and syphilis to rheumatism, asthma and other inflammatory diseases [12,13].

In the present report, the leaf extract of *Clerodendrum polycephalum* was tested for its *in vivo* antimalarial activity on infected albino mice using 3 models: suppressive, prophylaxis and curative. The suppressive activity on *Plasmodium berghei berghei* was found to be remarkable (Fig. 1); the % chemosuppression increasing with dose. The extract had higher % chemosuppression than standard drug chloroquine from 200 mg/kg to the highest dose 600 mg/kg (87.41-92.63% compared to 75.38%).

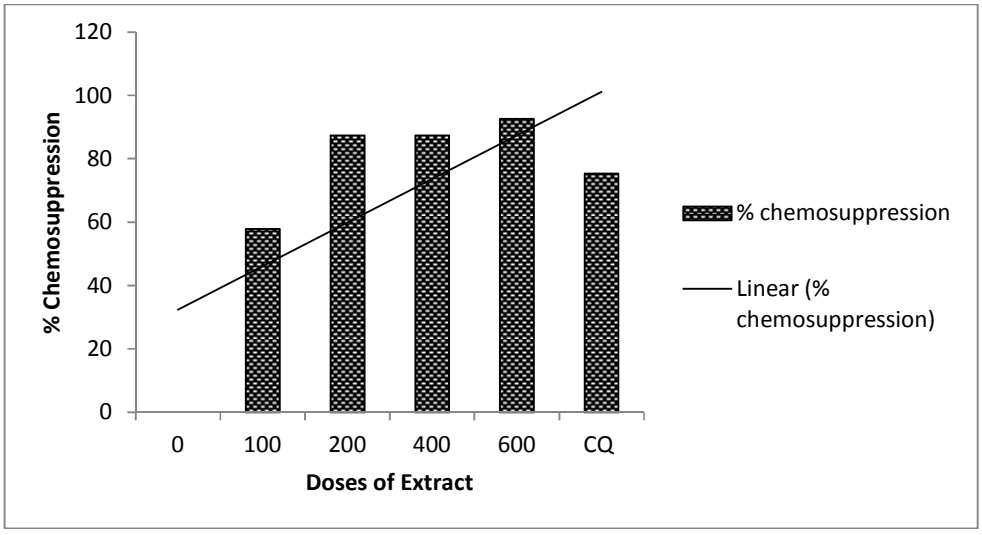

Fig. 1. The % parasitaemia and the % chemosuppression of *Clerodendron polycephalum* (suppressive test) on *Plasmodium berghei berghei*

The results of the prophylactic assay (Fig. 2) also showed remarkable potentials. Again, the extract compared favourably with the standard pyrimethamine exhibiting % chemosuppression of 64.73-91.56% as against 61% obtained for the standard. There was evident effectiveness of the extract also in the curative assay (Fig. 3); the % chemosuppression increasing up to day 5 for each dose which appeared to be the optimal potency period for the plant. In the curative assay the standard drug chloroquine showed higher activity than the extract.

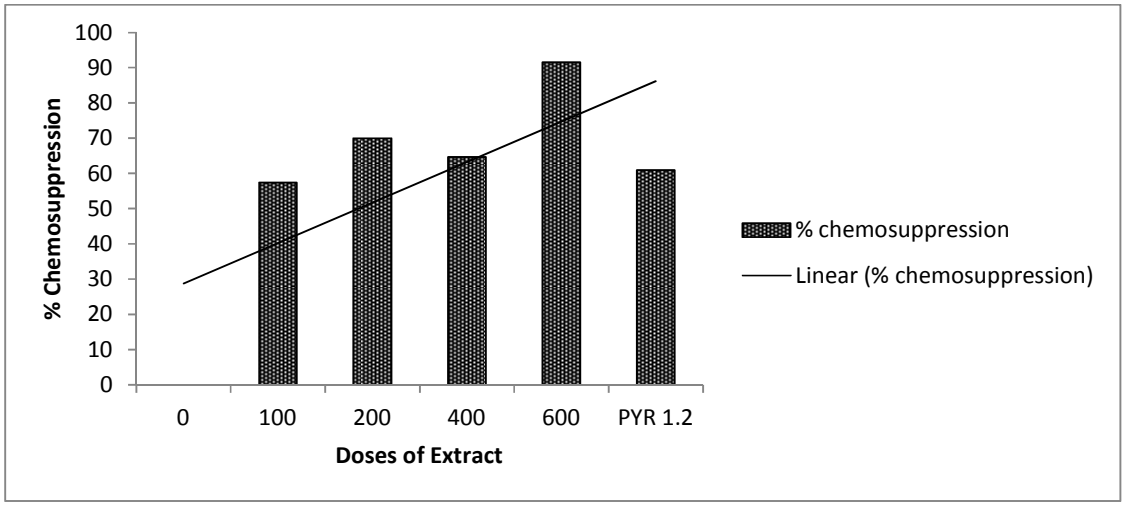

Fig. 2. The % parasitaemia and the % chemosuppression of *Clerodendron polycephalum* (prophylaxis test) on *Plasmodium berghei berghei*

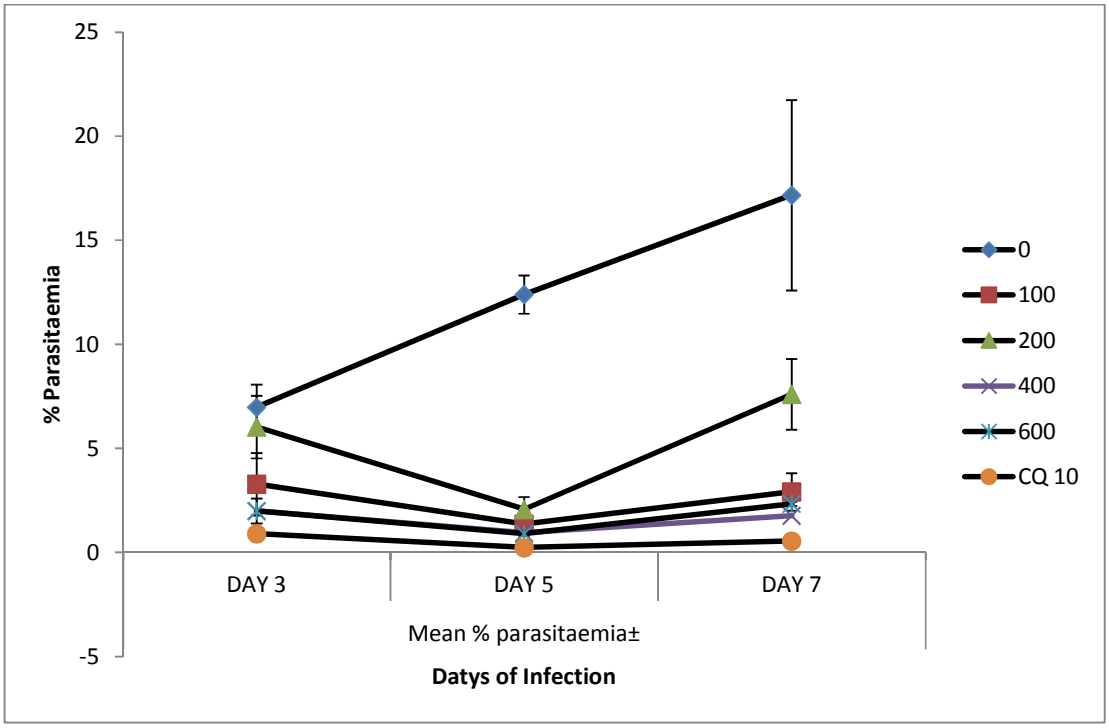

Fig. 3. The summary of % parasitaemia and the % chemosuppression of *Clerodendron polycephalum* (Curative test) on *Plasmodium berghei berghei*

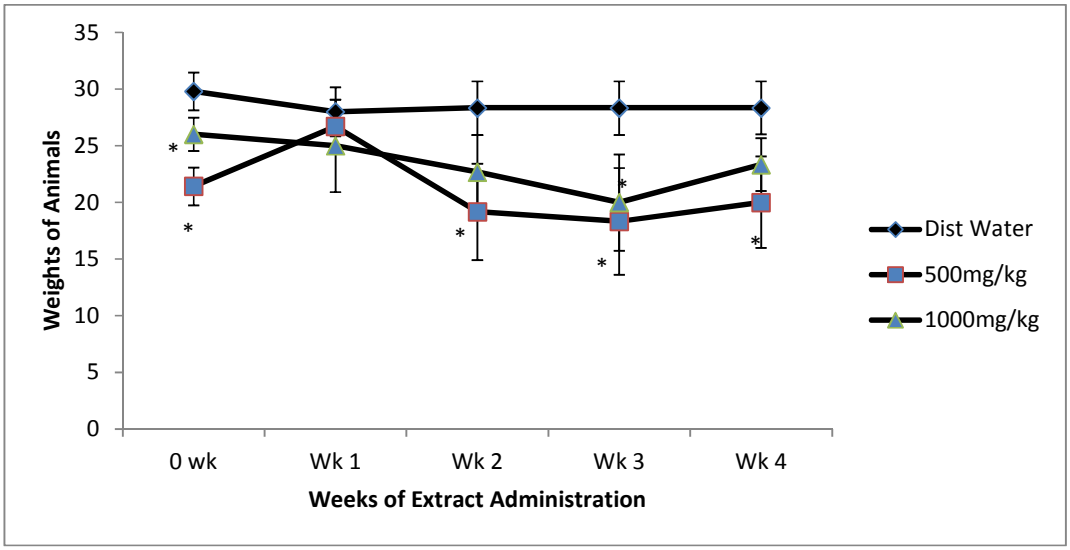

Fig. 4. The results of sub-chronic oral toxicity test of *Clerodendrun polycephalum* in mice

Research reports on the Clerodendrun genus denote the major presence of steroids as chemical constituents. Examples of this being β-sitosterol, γ-sitosterol, octacosanol, clerosterol, bungein, acteoside and betulinic acid. In addition are other constituents such as the terpenes (e.g. α-amyrin, β-amyrin, caryoptin) and the flavonoids (e.g.cynaroside, kaempferol, apigenin, 5-hydroxy-4,7dimethoxymethyl flavones [14]. Interestingly, the phytochemical study carried out [5] (confirmed by our unpublished results) on the crude extract revealed the presence of alkaloids and flavonoids, tannins, saponins among others. Alkaloids and flavonoids have proven to have antimalarial activities [15]. Phenolic compounds generally have diverse physiological effects including antiparasitic [16]. The overall antimalarial activity observed in this study could be attributed to the combined effects of these compounds.

Terpenes and flavonoids are noted to be very active anti-oxidants which play vital roles in ridding the body system of the derogatory effects of the inflammatory reactions that occur during infections. This is evident in the plasmodium infection in which several oxidants are involved in the biochemical reactions accompanying the pathological activity of the parasite in invading the erythrocytes [17].

The results of the acute toxicity assessment indicate safety in the use of the plant as the test animals survived the 5000 mg/kg dosing because an LD_{50} value greater than 5 g/kg is of no practical interest [18]. In addition, observations made from the acute oral toxicity test by the 10^{th} day include slight thinning of the fur in the anterior area and loss of appetite. At the end of the assay period, no mortality was observed.

The inability of the extract to completely cleared the parasites in infected animals may be due to the fact that sufficient active compounds had not accumulated enough. Overall, the plant extract was well tolerated by the animals. There were slight changes in body weight but which generally equated with the starting weight by the end of the test period. An indication of recovery from any toxic effect the extract might have had initially.

The values obtained for the haematological parameters (the Mean Corpuscular Volume, Mean Corpuscular Haemoglobin Concentration) also showed slight reduction compared with the control (Table 1). Altough, the extract significantly affected the RBC and PCV levels, but not to the level that could cause any physiological damage. The observed reduction may be due to haemolysis caused by osmotic changes in the blood system brought about by the presence of the extract. The WBC remained unchanged. This will indeed assist the animal to fight infection normally.

Table 1. Haematological profiles of the methanolic extract of *Clerodendron polycephalum* leaves in mice

Dose mg/kg	PCV	Hb value (g/dl)	RBC x 10^6 /mm^3	WBC x 10^4/ mm^3	MCV/μm^3	MCH pg	MCHC %
0	56.7±0.9	60.0±1.2	3.6±0.1	1.2±0.2	156.4±6.6	16.6± 0.4	105.9±2.2
500	45.7±1.2*	54.0±3.5*	2.9±0.2*	1.4±0.3	161.1±11.1	18.9±0.6*	118.0±4.7*
1000	45.3±1.8*	54.67±1.8*	3.0±0.01*	1.3±0.2	151.1±1 .9	18.2±0.4*	120.8±4.2*

**significant at P=.05 (p value ranged from 0 to 0.04)*

Key: PCV: Packed Cell Volume; Hb: Haemoglobin; RBC: Red blood cell count; WBC: White blood cell count; MCV: Mean corpuscular volume = (PCV/RBC) X 10; MCH: Mean corpuscular haemoglobin = (Hb/RBC) X 10; MCHC: Mean corpuscular haemoglobin concentration = (Hb/PCV) X 100

4. CONCLUSION

The leaves of *C. polycephalum* contain phytochemicals capable of suppressing and inhibiting the multiplication of *Plasmodium berghei berghei* thus can be used in ethnomedines as malarial remedy. The use of the plant should be considered as an alternative to conventional antimalarial drug. The study encouraged investigations into determination of specific active compounds. The study confirmed its traditional usage as antimalarial medicinal plant.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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