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Antiplasmodial Efficacy of *Anacardium occidentale* in Albino Mice Infected with *Plasmodium berghei*

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Abstract

Introduction: Resistance of malaria parasites by most malarial drugs prompted the search for other drugs that are effective against the parasite. In endemic nations of the world, medicinal plants are often used to treat malaria. Among such plants is *Anacardium occidentale* which in addition to treating malaria, the plant has traditionally been used to treat diarrhoea, dysentery, colonic pains, genital problems, venereal diseases, impotence, bronchitis, cough and syphilis-related skin disorder. This research aimed at exploring the efficacy of *A. occidentale* in the treatment of malaria.

Methods: The efficacy of *A. occidentale* was evaluated *in-vivo* in Swiss albino mice infected with *Plasmodium berghei* NK65. Blood samples of mice infected with *P. berghei* were tested against the plant extract for four consecutive days.

Results: The results revealed a significant reduction in the parasitaemia of the mice after treated with varying doses (400 mg/kg, 600 mg/kg and 800 mg/kg) of *A. occidentale* relative to the control groups. This revealed that tested doses of the plant extract produced various curative effects. *A. occidentale* exhibited high antimalarial properties of 80.66% and 80.69% curative at 600 mg/kg and 800 mg/kg doses respectively. However, low antimalarial property (54.20%) was observed at 400 mg/kg treatment.

Conclusions: This result shows that *A. occidentale* extract possess promising antimalarial activities which can be explored for malaria therapy.

Keywords

Anacardium occidentale, Efficacy, Antimalarial, *Plasmodium berghei*, Parasitaemia

List of Abbreviations

A. occidentale: Anarcadium occidentale; WHO: World Health Organization; CQ: Chloroquine; ACTs: Artemisinin Combination Therapies; IAMRAT: Institute of Advanced Medical Research and Training; UCH: University College Hospital; ACD: Acid Citrate Dextrose; RBC: Red Blood Cell; DNMRT: Duncan's New Multiple Range Test

Introduction

Malaria is a vector-borne disease transmitted by *Anopheles* mosquito in both humans and animals [1]. *Plasmodium* species, the aetiological agents include: *P. falciparum, P. vivax, P. malariae* and *P. ovale*. Malaria is vectored by female mosquitoes of the species *An. funestus, An. moucheti, An. gambiae,* and *An. arabiensis* [2,3]. In 2016, there were 216 million cases of malaria worldwide resulting in an estimated 731,000 deaths, approximately 90% of these cases and deaths occurred in Africa [4]. The disease is prevalent in tropical and some subtropical regions of Africa, Central and South America, Asia, and Oceania. The intensity and risk of malaria transmission in endemic areas vary significantly. For instance, more than 90% of clinical malaria and mortality occur mostly in sub-Saharan Africa [5]. In addition, highland (> 1,500 m) and arid areas (< 1,000 mm rainfall/year) typically have less malaria, although, these areas are prone to epidemic malaria if climatic conditions become favorable to mosquito development [4]. It has been estimated that about 40% of all fever episodes in sub-Saharan Africa are caused by malaria. In Nigeria, malaria is a major public health concern where it results in more morbidity and deaths than any country in the world [6]. About 97% of Nigeria population is at risk for malaria while only 3% of Nigeria's population that live in areas that are free of malaria [7]. The epi-

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demiological situation of malaria is worsening with the spread of drug resistance in the parasite and insecticide resistance in the vector. This significantly account for increasing malaria morbidity in sub-Saharan Africa by *P. falciparum* to the existing first line drugs such as chloroquine and sulfadoxine/pyrimethamine.

Currently, the biggest concern all over the globe is to treat patients with safe and effective medications and to avoid the parasites developing resistant against such medications [8]. Resistance to currently available antimalarial drugs has been confirmed in most of the human malaria parasite species especially *P. falciparum* and *P. vivax*. This resistance poses a threat to control and prevention of malaria, this necessitated the need to develop new drugs that are effective and affordable [9]. One of the drugs in which *P. falciparum* resistance has been reported in 2001 is Artemisinin [10]. This was observed in the border between Thailand and Myanmar, Artemisinin resistance became a major concern in 2008. Also, in 2009, *P. falciparum* resistant to ACTs was reported in patients in five regions and states in south-eastern Myanmar [10].

As a result of resistant malaria parasite strains and chemotherapy treatment failure, ethnomedicine has proven to be a veritable source of antimalarial treatment regimens or a template for the synthesis of novel antimalarial molecules [9]. Nigeria has rich flora diversity and many of the plant species are used by some indigenous people for medicinal purposes. A larger number of medicinal plants are used to treat malaria in the Southern part of the country where rain forests exist and originate a humid tropical climate, with ideal conditions for persistent malaria transmission all year round [11]. Some plant species are used for malaria treatment across all ethnic and cultural groups in the country, for example, *Alstonia boonei* (Apocynaceae), *Azadirachta indica* (Meliaceae) and *Cymbopogon citratus* (Poaceae).

Plants of the Meliaceae family are commonly used for malaria treatment in Nigeria, like the species *Azadirachta indica, Khaya senegalensis* and *Khaya grandifoliola. Azadirachta indica* is called “neem tree” and is also used in other African countries as a decoction against fever and/or malaria [11].

A research into the antimalarial plants may be a lead way for new affordable phytotherapies in malaria treatment especially among the less privilege who are mostly at risk of the devastating effects of malaria [11]. Therefore, the present research evaluates the antimalarial efficacy of *A. occidentale* in malaria treatment using *P. berghei* as an animal parasite model.

Methods

Plant collection and identification

Fresh green leaves were obtained from the stem of *A. occidentale* from the Federal University of Akure campus. The plant was identified by a plant taxonomist in the department of Biology, Federal University of Technology Akure, Ondo State.

Extraction of plant material

The leaves were air dried under the shade in the laboratory. The dry leaves were pound to a coarse powder in mortar and then to fine powder in blender. Extraction was carried out by dispersing 250 g of ground plant material in 2 liters of 96% ethanol for 72 hours and the preparation was stirred every 2 hours. The preparation was filtered with muslin cloth and the extract concentrated using a rotary evaporator at a temperature not more than 40 °C. The solvent free extract was concentrated using water bath.

Experimental animals

Thirty-five (35) Swiss albino mice with average weight 16-25 g obtained from Institute of Advanced Medical Research and Training (IAMRAT), University College Hospital, University of Ibadan were used for the study. The mice were distributed into 7 groups of five mice each (Table 1) and kept in plastic cages with sawdust as beddings and given food and water every day.

Table 1: Experimental plan for control and test animals.

Groups	Experimental plan	Remarks
Groups 1, 2, 3	Were infected with *Plasmodium berghei* and administered with *Anacardium occidentale* plant extract at 400, 600 and 800 mg/kg body weight respectively for 4 consecutive days.	Treatment
Group 4	Were infected with *Plasmodium berghei* and treated with 10 mg/kg of chloroquine phosphate solution for 4 consecutive days.	Standard control
Group 5	Were infected with *Plasmodium berghei* and only given 5 ml/kg of distilled water.	Negative control
Group 6	Were administered with 800 mg/kg body weight of *Anacardium occidentale* plant extract for 4 consecutive days.	Plant extract control
Group 7	Were neither infected nor treated.	Positive control

Table 2: Average percentage parasitaemia obtained from the mice in each treatment group.

Treatment with *Anacardium occidentale*	PARASITAEMIA				
	Time interval (Hours)				
	Before treatment	24 hours	48 hours	72 hours	96 hours
400 mg/kg	3.45 ± 1.7²a	2.18 ± 0.14[a]	2.03 ± 0.68[a]	1.68 ± 0.36[ab]	1.58 ± 0.36[ab]
600 mg/kg	5.43 ± 4.21[a]	2.12 ± 0.14[a]	2.03 ± 0.62[a]	1.91 ± 1.28[ab]	1.05 ± 0.56[a]
800 mg/kg	4.61 ± 2.37[a]	2.41 ± 1.25[a]	2.13 ± 1.03[a]	1.64 ± 0.90[ab]	0.89 ± 0.31[a]
Chloroquine 10 mg/kg	0.88 ± 0.44[b]	0.03 ± 0.03[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Infected untreated	0.70 ± 0.25[a]	5.35 ± 1.36[b]	10.39 ± 7.76[a]	4.56 ± 1.76[ab]	9.50 ± 1.77[b]

Superscripts [a-b] are means ± standard error represents 5 replicates. Mean values down the column with the same alphabet are not significantly different from each other at P > 0.05

Malaria parasite specimen

P. berghei (NK65) was acquired from the department of Parasitology, Institute of Advanced Medical Research and Training (IAMRAT), University College Hospital (UCH) Ibadan, Nigeria.

Inoculation of mice

A Swiss albino mouse (which served as the donor) was administered a standard inoculum of *P. berghei* intraperitoneally on day one. On the 7th day (after the parasite has stabilized in the host mouse) the mouse with a rising parasitaemia of about 20-35% was sacrificed and 0.1 ml of Acid Citrate Dextrose (ACD) was drawn into the syringe before blood was taken from the heart through cardiac puncture. The blood was diluted with isotonic saline to make inoculum for infecting experimental mice. The experimental mice were inoculated with 0.2 ml of diluted parasitized red blood cells specimen.

Determination of parasitaemia

Blood was obtained from the mice by cutting the tail from which thick and thin blood smears were prepared on sterile slides. The smears were fixed with methanol for 5 minutes with 10% Giemsa stain. The slides were observed under oil immersion objective lens of the compound microscope. The infected red blood cells and total number of red blood cells for each smear was recorded. The data obtained were used to determine the percentage parasitaemia and percentage curative were calculated using the formula below.

$$\% \text{ Parasitaemia} = \frac{\text{No of parasitized RBCs}}{\text{Total RBCs}} \times 100$$

$$\% \text{ Curative} = \frac{\text{Parasitaemia before treatment - parasitaemia after treatment}}{\text{Parasitaemia before treatment}} \times 100$$

Data analysis

The data obtained were analyzed and expressed as mean ± standard error. The mean and the level of significance for the differences between means of the data obtained were computed using Duncan's New Multiple Range Test (DNMRT) at P < 0.05

Results

Antiplasmodial activity of the plant extracts and chloroquine

The antiplasmodial activity of the plant extracts at graded doses of 400, 600 and 800 mg/kg body weight; the chloroquine at 10 mg/kg and negative control was presented in Table 2. On day 1 (24 hrs) and 2 (48 hrs), the percentage parasitaemia decreased in mice treated with *A. occidentale* at 400, 600 and 800 mg/kg and mice treated with 10 mg/kg of chloroquine. However, optimum activity of *A. occidentale* was recorded in all of the treatment groups in day 3 (72 hrs). At day 5 the curative test showed that there was no significant difference in the percentage parasitaemia at different doses of 400, 600, 800 mg/kg for the plant extract, and chloroquine at 10 mg/kg.

The mean parasitemia was highest in the infected untreated mice (negative control) while there was no parasitaemia in the red blood cells of mice treated with chloroquine, which is the standard control (Table 2). The plant extract cleared the parasitaemia to some extent in the various dosages at different intervals.

Percentage weight loss/gain of albino mice after treatment

The result showed significant weight loss in all the treated mice except mice treated with 800 mg/kg of *A. occidentale*, 10 mg/kg of chlorine and the positive control (mice neither infected nor treated). It was further observed that highest percentage weight loss (18.66%) was noted in mice treated with 800 mg/kg of *A. occidentale* for 24 hours while the least percentage weight loss (1.57%) was observed in mice treated with 10 mg/kg for 48 and 72 hours. Generally, it was noted that parasitaemia do not correlate with percentage weight loss. Carl Pearson's correlation showed no association between parasitaemia and weight loss (r = 0.295, n = 24 and p = 0.161). For instance mice infected with *P. berghei* but untreated showed the highest parasitaemia (10.39%) but has a low weight loss of 8.59%, while mice treated with 600 mg/kg of *A. occidentale* has parasitaemia of 1.05 and weight loss of 11.11% (Table 3). It was further

Table 3: Average percentage weight loss or gain after 96 hours of treatment.

Treatment at 24 hours interval	Parasitaemia %	Weight before treatment (g)	weight after treatment (g)	Weight gain/ loss (g)	%Weight gain/ loss
400mg					
24 hours	2.18	21.67	18.00	-3.67	-16.94
48 hours	2.03	21.67	20.33	-1.34	-6.18
72 hours	1.68	21.67	19.00	-2.67	-12.32
96 hours	1.58	21.67	18.33	-3.34	-15.41
600 mg					
24 hours	2.12	19.67	17.67	-2.00	-10.17
48 hours	2.03	19.67	16.67	-3.00	-15.25
72 hours	1.91	18.00	16.50	-1.50	-8.33
96 hours	1.05	18.00	16.00	-2.00	-11.11
800 mg					
24 hours	2.41	19.67	16.00	-3.67	-18.66
48 hours	2.13	19.67	17.66	-2.01	-10.22
72 hours	1.64	19.67	16.33	-3.34	-16.98
96 hours	0.89	21.00	21.00	0.00	0.00
Chloroquine					
24 hours	0.03	21.67	18.67	-3.00	-13.84
48 hours	0.00	21.67	21.33	-0.34	-1.57
72 hours	0.00	21.67	21.33	-0.34	-1.57
96 hours	0.00	21.67	21.67	0.00	0.00
infected untreated					
24 hours	5.35	19.33	16.33	-3.00	-15.52
48 hours	10.39	19.33	17.67	-1.66	-8.59
72 hours	4.56	19.33	18.00	-1.33	-6.88
96 hours	9.50	19.33	17.33	-2.00	-10.35
Uninfected untreated					
24 hours	0.00	22.33	23.33	1.00	4.48
48 hours	0.00	22.67	23.33	0.66	2.91
72 hours	0.00	22.67	23.67	1.00	4.41
96 hours	0.00	22.67	23.67	1.00	4.41

Negative sign indicates weight loss while positive sign indicates weight gain

observed that some of the mice regained their weight after treatment. For instance, mice treated with 800 mg of *A. occidentale* regained their weight from 16 g to 21 g at 24 to 96 hours of treatment respectively. Similar result was observed in mice treated with 10 mg of chloroquine, where the mice regained their weight from 18.67 to 21.67 g as the parasitaemia reduced from 0.03% to 0% at 96 hours treatment time (Table 3).

Percentage curative of *A. occidentale* at 24 hours interval

The curative result showed that chloroquine achieved 100% percentage curative at 48 hours of treatment (Table 4). Meanwhile, 600 mg/kg and 800 mg/kg of *A. occidentale* achieved 80.66% and 80.69% percentage curatives respectively at 96 hours of treatment. The least percentage curative (54.20%) was observed at 400 mg/kg for 96 hours of treatment. It was generally observed that the parasitaemia reduced while the percentage curative increased as the dose and time of treatment increased.

Discussion

Malaria is the major cause of health problem in tropical and developing countries of sub-Saharan Africa and South East Asia including India [12]. The emergence of widespread resistance of *Plasmodium* species to most antimalarial drugs has led to a more vigorous and concerted research in traditional use of plants for malaria treatment [13]. The results of this research revealed that *A. occidentale* exhibited potent antimalarial activity against *P. berghei*. This was noticeable as the parasitaemia reduced at different doses and time interval. This result agrees with the findings of [14] who reported that *A. occidentale* has the potential to relieve fever and cure malaria. This study provides a scientific evidence

Table 4: Parasitaemia and Percentage Curative of Mice Infected with *P. berghei* and Treated with Extract of *A. occidentale*.

Treatment	Parasitaemia Before Treatment	Parasitaemia at 24 Hours	Percentage Curative (%)	Parasitaemia at 48 Hours	Percentage Curative (%)	Parasitaemia at 72 Hours	Percentage Curative (%)	Parasitaemia at 96 hours	Percentage Curative (%)
***A. occidentale* 400 mg/kg**	3.45	2.18	36.81	2.03	41.16	1.68	51.30	1.58	54.20
600 mg/kg	5.43	2.12	60.96	2.03	62.62	1.91	64.83	1.05	80.66
800 mg/kg	4.61	2.41	47.72	2.13	53.80	1.64	64.43	0.89	80.69
10 mg/kg Chloroquine	0.88	0.03	96.59	0	100	0	100	0	100

for the claims.

The findings of the present research also conforms to the work of [13] who reported that the ethanolic leaf extract of *A. occidentale* were effective against malaria parasite. The *in vivo* study of the ethanolic extract of *A. occidentale* showed that the plant was effective at different dosage levels. The curative antiplasmodial tests showed that mice treated with *A. occidentale* at graded 400, 600, 800 mg/kg resulted in a decrease in parasitic load compared to the negative control.

The plant extract showed no significant difference ($p > 0.05$) against *P. berghei* in mice tested at all dosage levels from 24 hours to 96 hours of treatment compared to the mice in the untreated group. The percentage curative antiplasmodial activity of *A. occidentale* and chloroquine (standard control) at 10 mg/kg body weight from this study showed that chloroquine, the standard drug cleared the parasites by 100% compared to the percentage of clearance by plant extract that varies at graded doses of 400, 600 and 800 mg/kg against *P. berghei* infection. This agrees with the findings of [15] who reported total clearance of parasitaemia in the experimental mice treated with chloroquine, the author affirmed that the drug is still effective against malaria parasites that have not developed resistance yet. *A. occidentale* demonstrated curative effect of 54.20%, 80.66% and 80.69% at 400 mg, 600 mg and 800 mg respectively. This result showed that the drug is more effective at higher doses. However, the development of new drugs from the highly active natural products, which have already been discovered, is crucial in order to overcome the increasing resistance of *Plasmodium* to available antimalarial drugs. Therefore, there is a need to further phytoresearch especially on plants which have already been shown to have antimalarial activities.

Conclusion

The results of this study show that the leaf of *A. occidentale* is efficacious against *P. berghei* infection. This result has established the underlying principle for the traditional use of the *A. occidentale* in the malaria treatment, and demonstrated that medicinal plants which have reputation for antimalarial properties can be screened in order to ascertain their efficacy and determine their potentials as sources of new antimalarial drugs. However, the development of new drugs from the highly active natural products, which have already been discovered, is crucial in order to overcome the increasing resistance of *Plasmodium* to available antimalarial drugs. Therefore, there is a need to further phytoresearch especially on plants which have already been shown to have antimalarial activities.

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Conflict of Interests

The authors have no competing interests.

Ethics Approval and Consent to Participate

The ethic and consent concerning the use of mice for this research were waived by the Institute of Advanced Medical Research and Training, College of Medicine, University of Ibadan. Meanwhile, the number of mice used for this research was regulated by the Institute.

References

1. Ukaegbu CO, Nnachi AU, Mawak JD, Igwe CC (2014) Incidence of concurrent malaria and typhoid fever infection in febrile patients in Jos, Plateau State Nigeria. International Journal of Scientific and Technology Research 3: 157-161.
2. Kar NP, Nanda N, Bhatt RM, Sharma SN, Rana PK, et al. (2012) Prevalence and incrimination of Anopheles fluviatilis species (Diptera: Culicidae) in a malaria endemic forest area of Chhattisgarh state, central India. Parasite and Vectors 5: 215-220.
3. World Health Organization (2015) Fact Sheet: World Malaria Report.
4. World malaria report (2008) Geneva, World Health Organization. (WHO/HTM/GMP/2008.1).
5. World Health Organization (2014) World malaria report. Geneva.
6. World Health Organization (2017) World malaria report. WHO – Global Malaria Programme.
7. Nigeria Malaria Fact Sheet (2011) Prevalence of malaria in Nigeria.
8. Sinha S, Medhi B, Sehgal R (2014) Challenges of drug-resistant malaria. Parasite Journal 21: 61.
9. Abiodun O, Gbotosho G, Ajaiyeoba E, Happi T, Falade M, et al. (2010) In-vitro antiplasmodial activity and toxicity assessment of some plants from Nigerian ethnomedicine. Pharm Biol 49: 9-14.
10. World Health Organization (2014) Global malaria report: Status report on artemisinin resistance.
11. Adebayo JO, Krettli AU (2011) Potential antimalarials from Nigerian plants: A review. J Ethnopharmacol 133: 289-302.
12. Bahekar S, Kale R (2013) Herbal plants used for the treatment of malaria – a Literature review. Journal of Pharmacognosy and Phytochemistry 8192: 141-146.
13. Sha'a KK (2014) 'Antiplasmodial activity of aqueous and ethanolic extracts of Anacardium occidentale and Cymbopogon citratus', PhD thesis; University of Jos, Jos.
14. Odugbemi TO, Akinsulire OR, Aibinu IE, Fabiku PO (2007) Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria. Afr J Tradit Complement Altern Med 4: 191-198.
15. Soniran O, Idowu O, Ajayi O, Olubi I (2012) Comparative study on the effects of chloroquine and artesunate on histopathological damages caused by Plasmodium berghei in four vital organs of infected albino mice. Malaria Research and Treatment 22: 1-7.