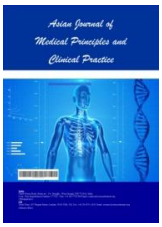

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Antiplasmodial Potential of Ethanolic Leaf Extract of *Jatropha curcas* against *Plasmodium berghei*

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Authors' contributions

This work was carried out in collaboration between both authors and both authors read and approved the final manuscript.

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ABSTRACT

Background: Malaria is one of the most several pathogenic diseases in endemic areas of the world, particularly in Africa. The causative parasites have developed resistance to orthodox drugs over the years, thus the need for herbal remedy.
Aim: This study is aimed at assessing the antiplasmodial potential of ethanolic leaf extract of *Jatropha curcas* against *Plasmodium berghei* in infected Swiss albino mice.
Methodology: Healthy leaves of *J. curcas* were harvested, dried and extracted using the soxhlet apparatus and ethanol as the solvent. The toxicity test was carried out the using standard method. Sixty Swiss albino mice obtained from the Federal University of Agriculture, Abeokuta, Nigeria were acclimatized for seven days and divided into six groups of ten each. Each mouse in groups 2 to 6 was inoculated intraperitoneally with infected blood suspension containing about $1x10^7$ *Plasmodium berghei* parasitized red blood cells on day zero while those in group 1 were not infected and this group served as the normal control group. Animals in group 2 were administered 0.2 mL normal saline, those in group 3 were administered Chloroquine diphosphate at 5 mg/kg body weight; those in groups 4, 5 and 6 were administered 100, 200 and 400 mg/kg body weight of *J. curcas* leaf extract respectively. All treatments were orally done twelve hourly for five consecutive

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days from when parasites were first seen in the infected animal blood. Parasitaemia count and packed cell volume (PCV) were determined using standard methods.
Results: *J. curcas* leaf extract was safe in rats at the tested oral doses (500–2000 mg/kg). There was no mortality within the study period. In the *in vitro* experiment, *J. curcas* prevented weight loss and reduced parasitaemia count in a dose-dependent manner. Its effect on PCV was only significant at a dose of 400 mg/kg body weight of the extract.
Conclusion: The present study revealed that ethanolic leaf extract of *J. curcas* possesses antiplasmodial potential against *P. berghei*. Maximum antimalarial efficacy of plant extracts and standard antimalarial drugs can be derived when dosage are completed.

Keywords: Antiplasmodial potential; chloroquine; Jatropha curcas; parasitaemia counts; Plasmodium berghei.

1. INTRODUCTION

Malaria is a vector-borne infectious disease that is widespread in tropical and subtropical regions. The term 'global change' is used to encompass all of the significant drivers of environmental change as experienced by hosts, parasites and parasite managers [1]. The antimalarial potential of compounds derived from plants has been proven to be effect. They include quinine, obtained from *Cinchona* species, and artemisinin, obtained from *Artemisia annua*. The selection of plants to be screened for antimalarial activity is made on the basis of the traditional reputation of particular plants for efficacy in the treatment of malaria. Scientists therefore, have embarked on a mission to survey the flora extensively to discover more and more potential plants have insecticidal properties [1].

Currently, there is a considerable increase in mortality caused by malaria due to the rapid spread of drug-resistant strains of *Plasmodium falciparum* and *Plasmodium berghei*. The asexual erythrocyte cycle of human malaria parasite causes severe forms of the disease [2]. Invasion of an individual parasite into a red blood cell initiates the cycle; approximately 48 hours later releases of 16 - 32 daughter parasites terminate the cycle to spread the infection. In Southeast Asia alone, 100 million malaria cases occur every year and 70% of these are reported from India [3]. The use of chloroquine (CQ) to prevent and treat *P. berghei* malaria has led to the wide-spread appearance of CQ-resistant strains against *P. berghei* throughout the affected regions. The resistance has at the same time increasingly extended to other available antimalarial drugs [4].

Jatropha curcas L. (physic nut) is a species of flowering plant in the spurge family, Euphorbiaceae. It is native to the American tropics most likely Mexico and Central America [5]. It is commonly known as a biodiesel fuel plant. In Nigeria, it is called 'Lapalapa' by the Yorubas, 'Cinidazugu' by the Hausas, 'Olulu-idu/uru' by the Igbos, 'Omangba' by the Iyedes in Benue State and 'Itiakpa' by the Urhobos in Delta State. It is now widely cultivated in both tropical and sub-tropical regions around the world [6,7]. It produces flowers and fruits throughout the year. The seeds contain between 27 and 40% oil which can be processed to produce a high-quality biodiesel fuel useable in a standard diesel engine [8].

J. curcas has been reported to have a lot of health benefits because of its wide range of medicinal uses [9]. The name *Jatropha curcas* meaning (Doctor's nutrient) was related to its numerous medicinal purposes [10]. The leaves are regarded as antiparasitic, they are applied to scabies, rubefacient for paralysis, rheumatism and also applied to hard tumor [11]. The sap from the leaves can be used on bee or wasp sting. The leaves, when pounded can be applied on the eye of a horse to scare flies from it especially in India. The leaves contain apigenin, vitexin and ansovitexin which when combined with other factors enable them to be used against muscular pains [9]. The oil from *J. curcas* seeds is used in helping with rashes and parasitic skin diseases [12]. When the oil is mixed with benzyl benzoate, it becomes effective against scabies and dermatitis [13]. The oil from the seed can also be applied to soothe rheumatic pain. Jatropha kernel oil together with about 36% linoleic acid is a possible interest for skin care industry. The use of the oil may cause premature abortions [14]. The sap from the bark is used to dress bleeding wounds and ulcers and can also be used to stop bleeding. The sap from the leaves is also used as an application for the treatment of pile. The latex is also applied

topically to bee and wasp stings, boils and sores. The latex is also used to treat tooth ache, ringworm. Latex is use to dress sores, ulcers and inflamed tongues [9]. Airaodion and Ogbuagu [15] have reported the abortifaceous properties of *J. curcas* leaf extract in female Wistar rats. The plant has also been speculated to possess antimalarial potential. This study is therefore aimed at assessing the antimalarial potential of *J. curcas* against *Plasmodium berghei.*

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Fresh and healthy leaves of *J. curcas* free from disease were harvested from Odo-Ona area of Ibadan, Nigeria and were identified by a botanist. They were washed in running water to remove contaminants. They were air-dried at room temperature in open laboratory space for 14 days and milled into powder using an electric blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the method described by Airaodion et al. [16,17]. About 25 g of the powder was packed into the thimble of the soxhlet extractor. 250 mL of ethanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle solvent was heated using the heating mantle and began to evaporate moving through the apparatus to the condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the ethanol was evaporated in a rotary evaporator at 35^{o}C with a yield of 2.77 g which represents a percentage yield of 11.08%. The extract was preserved in the refrigerator at 4^{o}C for further analysis.

2.2 Oral Acute Toxicity Studies

Oral acute toxicity study was carried out according to the method described by Airaodion et al. [18]. Twenty-five rats were divided into five groups of five rats per group. Group A was given distilled water (10 mL/kg) while groups B, C, D and E were separately given 500, 1000, 1500, and 2000 mg/kg body weight of *J. curcas* extract respectively. Treatments were administered orally by gastric intubation. The animals were observed for 24 hours post treatment for signs of toxicity and then 48 hours for possible death.

2.3 Parasite Inoculums

Plasmodium berghei NK65 strain infected erythrocytes were obtained from a donor-infected mouse maintained at the Department of Veterinary Microbiology and Parasitology, Federal University of Agriculture, Abeokuta, Nigeria. The inoculum was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and then diluting with normal saline.

2.4 Experimental Animal and Curative Test

Sixty (60) Swiss albino mice weighing between 20 and 25 g were obtained from the Animal House of Federal University of Agriculture, Abeokuta, Nigeria. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into six groups of ten mice each. In order to evaluate the curative potential of the crude extract, methods described by Airaodion et al. [19] were adopted. Each mouse in the treatment group (groups 2 to 6) was inoculated intraperitoneally with infected blood suspension (0.2 mL) containing about 1x10^{7} *Plasmodium berghei* parasitized red blood cells on day zero while those in group 1 were not infected and this served as the normal control group. Animals in group 2 were administered 0.2 mL normal saline (negative control), those in group 3 were administered Chloroquine diphosphate (standard antimalarial drug) at 5 mg/kg body weight (positive control), those in groups 4, 5 and 6 were administered 100, 200 and 400 mg/kg of *J. curcas* extract respectively. All treatments were orally done twelve hourly for five consecutive days from when parasites were first seen in the infected animal blood. Four days after the treatment was stopped, the animals were weighed and sacrificed.

2.5 Parasitaemia Count

The method described by Airaodion et al. [1] was used to determine the parasitaemia counts. Briefly, on each day of treatment and post-treatment, a drop of blood was collected from each mouse for parasitaemia screening by tail nip. The blood collected was placed on a slide and smeared to make a thick film, fixed with ethanol and stained with Giemsa stain. When dried, the film was microscopically viewed by adding a drop of immersion oil and viewing it under x100 magnification of the microscope. The parasitaemia density was examined by counting the parasitized red blood cell.

2.6 Determination of Packed Cell Volume

The method described by Airaodion et al. [20] was used to determine the packed cell volume (PCV). Briefly, capillary tubes were filled with blood to about 1 cm or two-third (2/3) of its length and the vacant end of each tube was sealed with plasticin to protect the blood from spilling. The tubes were placed in haematocrit centrifuge with a sealed side towards the periphery and then centrifuged for 5-6 minutes. The packed cell volume was read directly from haematocrit reader [20].

2.7 Statistical Analysis

Data were subjected to analysis using Microsoft Excel and presented as mean ± standard deviation.

3. RESULTS

3.1 Acute Toxicity Studies

Ethanolic leaf extract of *J. curcas* was safe in rats at the tested oral doses (500–2000 mg/kg). There was no mortality within the study period. However, there were behavioral changes such as depression, reduced motor activity and ataxia.

3.2 *In vitro* Studies

The effect of *J. curcas* on body weight change, PCV and parasitaemia counts are presented in Figs. 1-3 respectively.

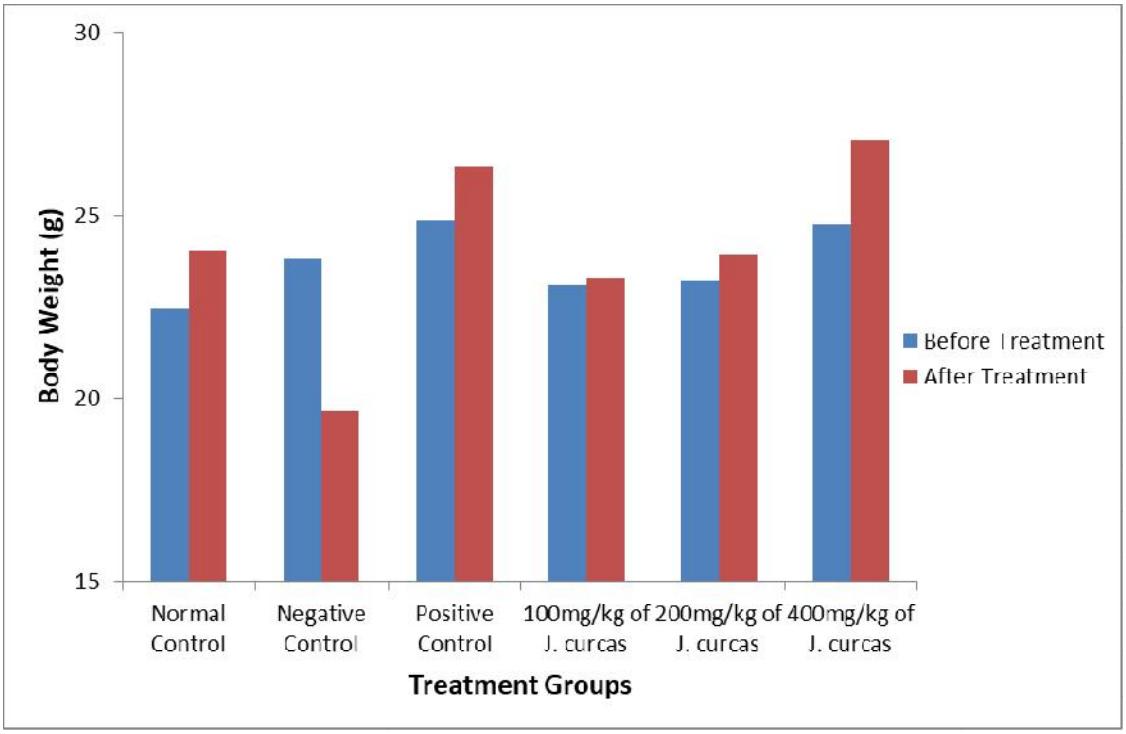

Fig. 1. Effect of ethanolic leaf extract of *J. curcas* on body weight of *P. berghei*-infected Mice

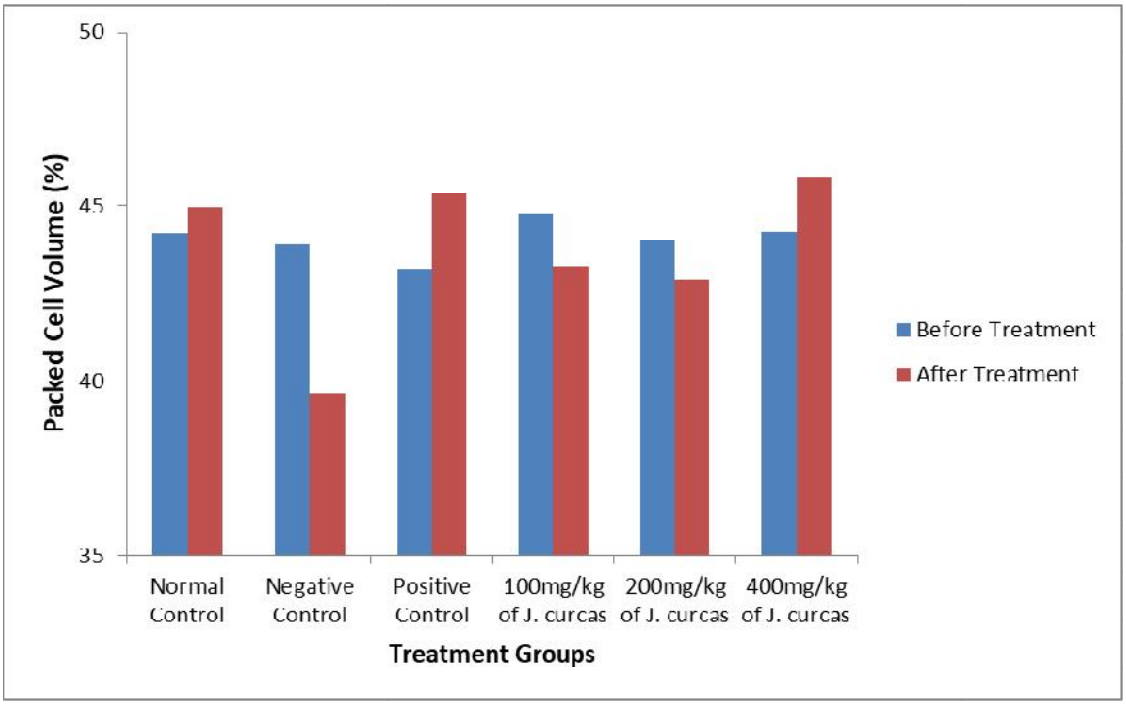

Fig. 2. Effect of ethanolic leaf extract of *J. curcas* on packed cell volume of *P. berghei*-infected Mice

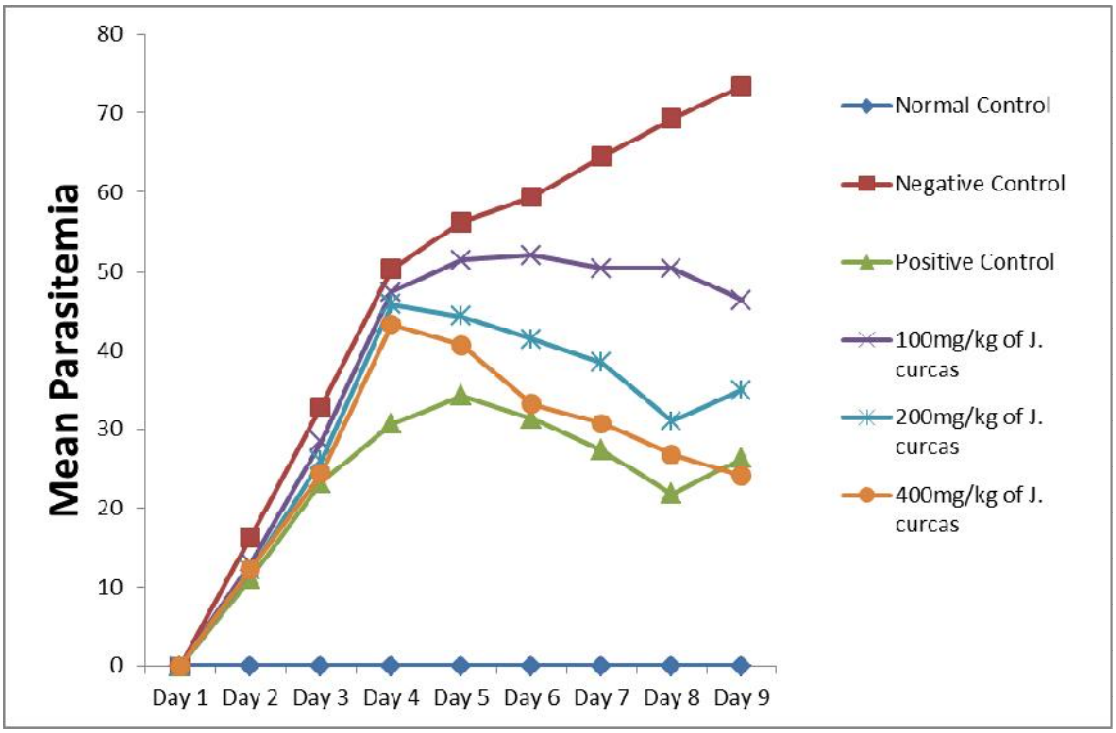

Fig. 3. *In vivo* antiplasmodial activity of ethanolic leaf extract of *J. curcas* against *Plasmodium berghei* in infected mice: Each point represents mean with n=10

4. DISCUSSION

There is a considerable increase in mortality caused by malaria due to the rapid spread of drug-resistant strains of *Plasmodium falciparum* and *Plasmodium berghei* [19]. Many antimalarial agents have been developed and used in the treatment of malaria, with significant cases of drug failures and the attendant side effects associated with these agents. The parasites have developed resistant to orthodox drugs over the years, thus the need for herbal remedy. Majority of the rural dwellers depend on traditional medicine as a source of primary health care including malaria treatment [20].

The result of the acute toxicity test of this study showed that *J. curcas* leaves is not toxic to health as no mortality was recorded after 48 hours of administration. The change in behavioural conduct of animals observed might be an indication that consumption of *J. curcas* leaves in high amount could lead to agitation or depression.

Results from this study indicated that the body weight of the infected but untreated mice (negative control) significantly reduced after 4 days of infection (Fig. 1). Treatment with 100 mg/kg, 200 mg/kg, 400 mg/kg body weight of *J. curcas* leaf extract showed weight gain after 4 days of treatment. However, the weight gain was only significant in animals treated with 400 mg/kg of *J. curcas* extract. This is suggestive that the extract is potent in combating weight loss sequel to malaria infection. Weight gain was also observed in the group treated with 5 mg/kg chloroquine (positive control). This is similar to the findings of Airaodion et al. [1,20] who reported the antiplasmodial potential of leaf-extracts of *Carica papaya* and *Vernonia amygdalina* respectively in *Plasmodium berghei*-infected mice. Body weight loss and fever are some features of malaria infection [19]. Therefore, a potent antimalarial plant should be able to prevent body weight loss in infected mice [21]. In the present study, the extract of *J. curcas* was observed to have prevented body weight loss linked with elevation in parasitaemia level.

Anaemia has been reported as one of the symptoms of malarial infection in mice [19]. Anaemic condition has been reported to be sequel to haemolysis [22,23]. From the results of this present study, the packed cell volume (PCV) of *P. berghei* infected but untreated mice (negative control) showed significant decrease in PCV after 4 days of infection (Fig. 2). This result revealed that *P. berghei* infection significantly reduced the red blood cells of animals. Treatment of infected mice with 100 mg/kg and 200 mg/kg of *J. curcas* leaf extract was unable to reverse the effect of *P. berghei* infection on the PCV of animals. However, treatment of infected mice with 400 mg/kg of *J. curcas* leaf extract as well as 5 mg/kg of chloroquine (positive control) showed a significant increase in PCV after 4 days of treatment (Fig. 2). This is indicative that treatment with this dose of extract (400 mg/kg) has the propensity to ameliorate the effect of *P. berghei* infection on the PCV of infected animals. This dose of extract competes favourably with the group treated with standard antimalarial drug (chloroquine) in this study.

Parasitaemia count is the primary indicator used in determining the degree of malarial infection. In this study, no noticeable difference was observed in the parasitaemia level of all the infected mice after 3 days of treatment. However, the effect of treatment became noticed after 4 days of therapy. This might be an indication that using *J. curcas* leaf extract for 3 days or less might not yield significant antimalarial effect. It could also mean that usage of antimalarial drugs prescribed for 4 days might not yield the maximum result if the dosage is not completed [19].

The result of this study also indicates that extract of *J. curcas* leaf extract reduced average daily parasitaemia level of infected mice in a dose-dependent manner with 400 mg/kg yielding a more reduced parasitaemia level and competing favourable with chloroquine, the standard antimalarial drug used in this study. This is in agreement with the findings of Offoumou et al. [24] who reported the antiplasmodial activity and phytochemical screening of crude extracts of *Jatropha curcas*, *Parkia bicolor* et *Vernonia amygdalina,* three traditional plants of Ivorian pharmacopeia. The result of this study is also consistent with the report of Abiodun et al. [25] who investigated the *in vitro* antiplasmodial activity and toxicity assessment of some plants from Nigerian ethnomedicine.

The antiplasmodial property of this plant extracts may be attributed to presence of some phytochemicals which might have conferred some protective/antioxidative effect against oxidative stress induced in the host parasitized red blood cells (RBCs) by the malarial parasite [26,27]. In malarial infection, oxidant damage to the erythrocyte membrane has been reported

with evidences revealing a causal relationship between haemichrome production and band 3 aggregations in oxidatively stressed RBCs [28]. This relationship could account for the deposition of band 3-specific autologous IgG and consequent deposition of fragments of complement C3c. Thus, leading to alterations of the surface of the infected RBCs and subsequent phagocytosis by macrophages [26,28]. Previous study has revealed that *J. curcas* leaves are rich in phytochemical compositions [24]. This could justify the antimalarial activities exhibited by the plant extract.

Similar studies have reported that *Jatropha tanjorensis* [28] and *Jatropha gossypifolia* [29] possesses antiplasmodial potential.

5. CONCLUSION

The present study revealed that ethanolic leaf extract of *J. curcas* possesses antiplasmodial potential against *P. berghei*. Maximum antimalarial efficacy of plant extracts and standard antimalarial drugs can be derived when dosage are completed.

CONSENT

It is not applicable

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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