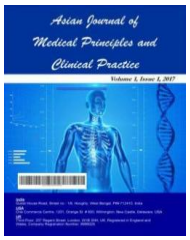

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Antiplasmodial Potency of Ethanolic Leaf Extract of *Carica papaya* against *Plasmodium berghei* in Infected Swiss Albino Mice

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Authors' Contributions

This work was carried out in collaboration with all authors. Author AIA conceptualized and designed the study, and also wrote the manuscript. Author EOA managed the analyses of the study. Authors EOO and JAE managed the literature searches. Author UO wrote the protocol and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: Malaria is still considered a major public health problem in developing countries. The malaria parasite has develop resistant to orthodox drugs over the years, thus need for herbal remedy.
Aim: This study is aimed at investigating the antiplasmodial potency of ethanolic leaf extract of *Carica papaya* against *Plasmodium berghei* in infected Swiss albino mice.
Methods: Fresh and health leaves of *C. papaya* free from disease were harvested from the Institute of Agricultural Research and Training, Ibadan. They were air dried, milled into powder and extracted using soxhlet apparatus and ethanol as the solvent. Thirty Swiss albino mice weighing obtained from the Federal University of Agriculture, Abeokuta, were acclimatized for seven (7) days and divided into six groups. Each mouse in groups 2 to 6 was inoculated intraperitoneally with infected blood suspension containing about $1x10^7$ *Plasmodium berghei* parasitized red blood

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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, 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 of the ethanolic leaf extract respectively. All treatments were orally done once per day for five consecutive days from when parasites were first seen in the infected animal blood. Parasitemia Count and PCV were done using standard methods.

Results: *C. papaya* extract exhibited antimalarial properties especially at 200 and 400 mg/kg and the results were not different from that of chloroquine.

Conclusion: The result of this present study confirmed that ethanolic leaf extract of *C. papaya* which displayed good activities against *P. berghei* are suitable for their traditional use in the treatment of malaria fever.

Keywords: Carica papaya; antiplasmodial potency; Plasmodium berghei; ethanolic leaf extract; swiss albino mice.

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 is proven by examples such as quinine, obtained from Cinchona species, and artemisinin, obtained from Artemisia annua [1]. The selection of plants to be screened for antimalarial activity is done on the basis of 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 the human malaria parasite causes severe forms of 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 South East 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].

Carica papaya belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases [5,6]. Originally derived from the southern part of Mexico, *C. papaya* is a perennial plant, and it is presently distributed over the whole tropical area. In particular, *C. papaya* fruit circulates widely, and it is accepted as food or as a quasi-drug. Many scientific investigations have been conducted to evaluate the biological activities of various parts of *C. papaya*, including fruits, shoots, leaves, rinds, seeds, roots or latex. The leaves of *C. papaya* have been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, à-tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates [7].

The hypoglycemic effect of ethanolic extract of papaya in alloxan-induced diabetes has been reported [8]. Fruit and seed extracts have pronounced bactericidal activities [9]. Leaves have been poulticed into nervous pains, elephantoid growths and it has been smoked for asthma relief amongst tropical tribal communities. Moreover, *C. papaya* leaf juice is consumed for its purported anti-cancer activity by people living on the Gold Coast of Australia, with some anecdotes of successful cases being reported in various publications. *C. papaya* leaf extracts have also been used for a long time as an aboriginal remedy for various disorders, including cancer and infectious diseases.

C. papaya contains two important biologically active compounds viz., chymopapain and papain which are widely used for digestive disorders [10]. It showed that papaya derived papain, caricain, chymopain, and glycerin endopeptidase can improve acidic pH conditions and pepsin degradation. Other active compounds of *C. papaya* are lipase, a hydrolase, which is tightly

bonded to the water-insoluble fraction of crude papain and is thus considered as a "naturally immobilized" biocatalyst [11]. According to the folk medicine, papaya latex can cure dyspepsia and also applicable for external burns and scalds. Seeds and fruits are excellent antihelminthic and antiamoebic [12]. Dried and pulverized leaves are sold for making tea; also the leaf decoction is administered as a purgative for horses and used for the treatment of genetic-urinary system. This present study sought to investigate the antiplasmodial potency of ethanolic leaf extract of *C. papaya* against *P. berghei* in infected Swiss albino mice.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Fresh and health leaves of *C. papaya* free from disease were harvested from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan and were identified by a botanist. They were washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory space for 14 days and milled into powder using an electronic blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the method described by Airaodion et al. [13,14]. 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 35ºC with a yield of 2.98 g which represents a percentage yield of 11.92%. The extract was preserved in the refrigerator until when needed.

2.2 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 parasitemia and the erythrocytes count of the donor mouse and then diluting with normal saline.

2.3 Experimental Animal and Curative Test

Thirty (30) 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 five mice each. In order to evaluate the curative potential of the crude extract, methods described in literature were adopted Airaodion et al.[15] Akuodor and Idris [16]. 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 the ethanolic leaf extract respectively. All treatments were orally done once per day 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.

2.4 Parasitemia Count

On each day of treatment and post treatment, a drop of blood was collected from each mouse for parasitemia 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 parasitemia

density was examined by counting the parasitized red blood cell [15,16].

2.5 Determination of Packed Cell Volume

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 sealed side towards the periphery and then centrifuge for 5-6 minutes. The packed cell volume was read directly from haematocrit reader [15,16].

2.6 Statistical Analysis

Data were subjected to analysis using Microsoft Excel.

3. RESULTS AND DISCUSSION

The development of an affordable Artemisinin-based Combination Therapy (ACT) or an alternative cost-effective antimalarial drug is imperative in the rural areas where majority of the people are poor. Many scientists are now even turning towards herbs to seek for answers to drug resistance. Plants used in treatment of diseases are said to contain active phytochemicals some of which are responsible for the plants' characteristic adours, pugencies and color while others give virtues as food, medicinal or poisonous [17].

The result of the effect of ethanolic leaf extract of *C. papaya* on body weight of *Plasmodium berghei*-infected mice is shown in Fig. 1. The body weight of the infected untreated mice (negative control) and infected treated with 100 mg/kg of *C. papaya* showed significant weight lost after 4 days post treatment. On the other hand, the infected mice treated with 200 mg/kg, 400 mg/kg of *C. papaya* as well as those treated with 5 mg/kg chloroquine (positive control) showed weight gain after 4 days of treatment. The weight gain in these groups is not different when compared with uninfected animals (normal control). This is similar to the result of Airaodion et al. [18] who treated *Plasmodium berghei*-infected mice with ethanolic leaf extract of *Vernonia amygdalina*. Anemia, body weight loss and body temperature reduction are the general features of malaria infected mice [19]. So an ideal antimalarial agents obtained from plants are expected to prevent body weight loss in infected mice [20]. In the present study, extract of *C. papaya* significantly prevented weight loss associated with increase in parasitemia level.

Fig. 1. *Carica papaya* plant [8]

The effect of ethanolic leaf extract of *C. papaya* on packed cell volume (PCV) of *Plasmodium berghei*-infected mice is shown in Fig. 2. The PCV of *P. berghei* infected untreated mice (negative control) and infected treated with 100 mg/kg of *C. papaya* showed significant decrease in PCV after 4 days of treatment. On the other hand, the infected mice treated with 200 mg/kg, 400 mg/kg of *C. papaya* leaf extract as well as those treated with 5 mg/kg chloroquine (positive control) showed significant increase in PCV after 4 days of treatment. The significant increase in level of PCV and body weight in mice treated with *C. papaya at* 200 and 400 mg/kg when compared with the negative control group is an indication of ameliorating potentials of the plant extract on the anaemia induced by the malarial infection. This result contradicts the study of Airaodion et al. [18] who reported a decrease in the PCV of animals after treatment with with ethanolic leaf extract of *Vernonia amygdalin*.

The average daily parasitaemia level of the *P. berghei* in infected mice treated with ethanolic leaf extract of *C. papaya* is shown in Fig. 3. The average daily parasitaemia of infected mice treated, respectively, with chloroquine, 400 mg/kg and 200 mg/kg leaf extract of *C. papaya* significantly ($P<0.05$) reduced when compared with control group. However there is no significant ($p>0.05$) difference in the level of parasitaemia in infected mice treated with 100 mg/kg leaf extract of *C. papaya* as compared with the control group. This result is in agreement with the reprot of Airaodion et al. [18] who treated *Plasmodium berghei*-infected mice with ethanolic leaf extract of *Vernonia amygdalina*. It is also in agreement with the study of Longdet and Adoga [21] who reported the effect of methanolic leaf extract of *Carica papaya* on *Plasmodium berghei* infection in albino mice. Several studies of the qualitative phytochemical analysis of *C. papaya*

leaves showed the presence of alkaloid, flavonoid, Saponin, Tannin and Glycosides [21-24]. Flavonoids have been reported to have exhibited significant *in vitro* antimalarial activity against *P. falciparum* [25]. This could justify the antimalarial activities exhibited by the plant extract.

The 400 mg/kg, 200 mg/kg and 100 mg/kg ethanolic leaf extracts showed a dose dependent and progressive reduction in parasitaemia with time. This finding agrees well with earlier reports of studies using different solvents. Antiplasmodial activity was observed in the ethyl acetate crude extract of *C. papaya* against *P. falciparum* [26]; administration of aqueous leaf extract of *C. papaya* significantly (< 0.05) decreased parasite load in mice and enhanced their survival [27]; methanolic extract of *C. papaya* at 100, 200 and 400mg/kg body weight gave significant suppression (p<0.05) of parasitemia following five days administration in established infection [21]. This is a very promising feature in the potentials of *C. papaya* as an antimalarial agent. Good enough, the antimalarial effect demonstrated by *C. papaya* leaf extract compete well with chloroquine treatment. Chloroquine has been used as the standard antimalarial drug because of its established activities on *P. berghei* [28, 29].

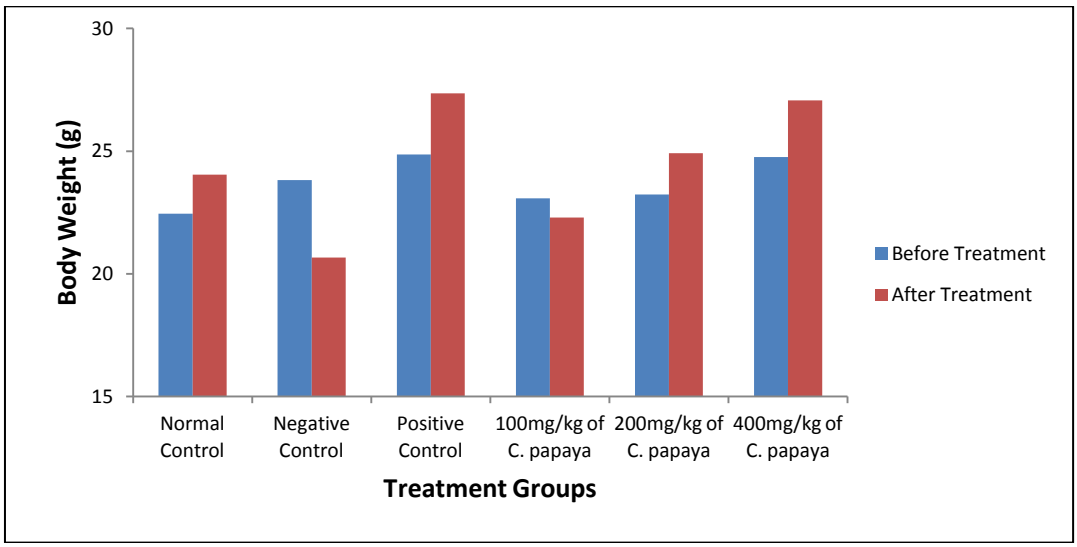

Fig. 2. Effect of ethanolic leaf extract of *C. papaya* on body weight of *P. berghei-i*nfected mice values are presented as mean with n = 5

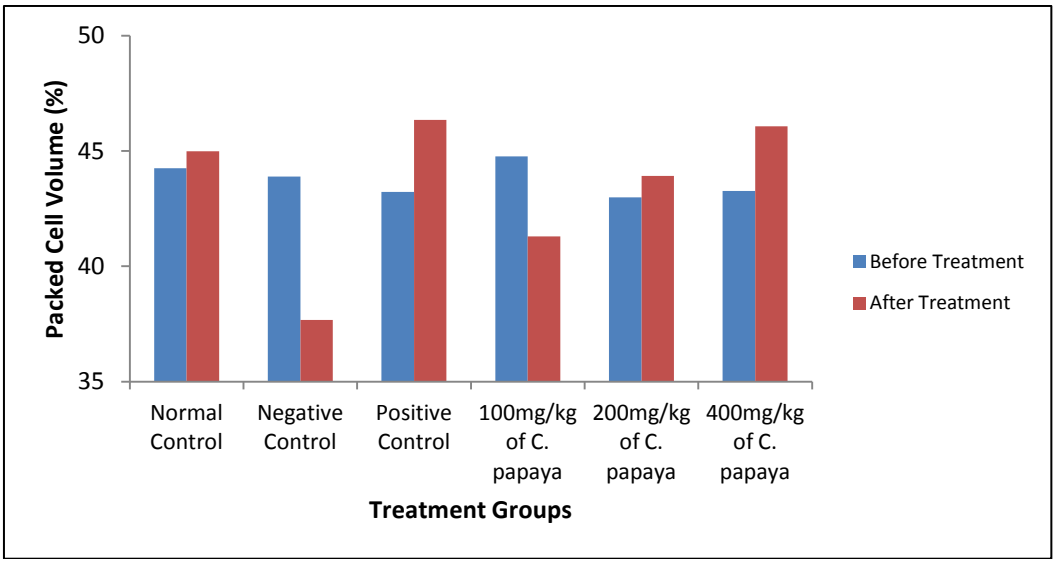

Fig. 3. Effect of ethanolic leaves extract of *C. papaya* on packed cell volume of *P. berghei*-infected mice

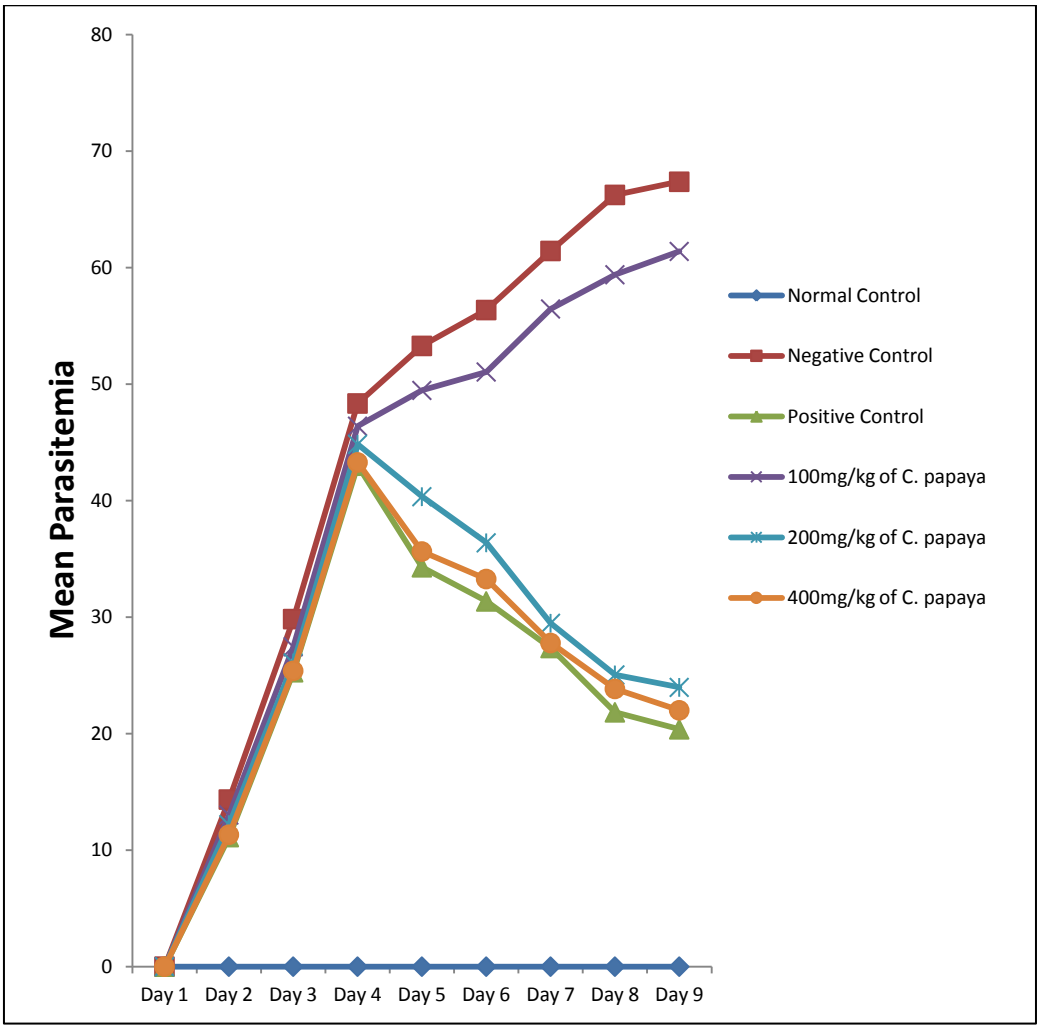

Fig. 4. *In vivo* antiplasmodial activity of ethanolic leaf extract of *C. papaya* against *Plasmodium berghei* in infected mice: Each point is a Mean±SD with n=5

4. CONCLUSION

Increasing the global spread of multi-drug resistant malaria parasite showed that there is a need for new chemotherapeutic agents to combat malaria. Development of new active and safe drugs for the community is therefore an urgent need. Towards this goal, research into new antimalarial drugs from natural products, traditional healers use parts of many plants for the treatment of several pathologies, including malaria, and have done so for centuries. The result of this present study confirm that extracts from leaves of *C. papaya* which displayed good activities against *P. berghei* are suitable for their traditional use in the treatment of malaria fever.

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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