Original Research Article

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The *in vivo* anti-plasmodium activity of *Garcinia kola* Heckel

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Abstract

Malaria is a tropical disease and a public health burden in parts of Africa, Asia, South and Central America. It is caused by protozoan parasite of the genus *Plasmodium*. Antimalarial drug resistance has become one of the greatest challenges against malaria control. In this regard, traditional medicine, particularly plant based antimalarial products that are readily available and cheap could be considered as alternatives if they have demonstrable antimalarial activity. This study investigated the antimalarial activity of ethanolic extract of *Garcinia kola* seed in mice. Mice were infected with *Plasmodium berghei* and treated with *Garcinia kola* extract. Chloroquine was used as the reference drug. The results showed a significant reduction ($p \leq 0.05$) in percentage parasitaemia in the infected mice treated with *Garcinia kola* extract. Packed cell volume (PCV), white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb) and platelet count of the infected and uninfected mice were also investigated. The results showed that the animals that were not infected and treated, the animals that were infected and treated with *Garcinia kola* extract, the animals that were infected and treated with chloroquine and the animals that were infected and treated with chloroquine and *Garcinia kola,* were all healthy and not anemic. There was a decrease in the packed cell volume and hemoglobin of the animals that were infected but not treated. Our findings suggest that *Garcinia kola* has a potential as an antimalarial agent, however more experiments are needed before it can be put to use.

Keywords: *Garcinia kola*, Antimalarial, Plasmodium berghei, in vivo, Heamatology, Plant extracts

1. Introduction

Malaria is an infectious disease caused by the parasite, *Plasmodium*, which infects red blood cells. It is one of the leading infectious diseases in many tropical regions in Asia, South and Central America and Africa. In West Africa, including Nigeria, transmission occurs all year round. Malaria was responsible for over 1.2 million deaths in 2010 alone. In 2015, an estimated 355 million persons were at a risk for malaria globally according to a 2016 report of the World Health Organization [1]. In Sub-Saharan Africa, about 90% of all malaria deaths occur among children under 5 years of age [2, 3]. Current estimate shows that a child dies of malaria every 30 seconds in Africa. In Nigeria, the disease is a major health problem with stable transmission throughout the country. It accounts for about 50% of outpatient consultation, 15% of hospital admission and is the prime amongst the top three causes of death in the country [3]. More importantly, it is a social and economic problem which consumes about US $5 million in various control attempts [3].

Around the world, malaria is becoming more resistant to a number of antimalarial drugs that are currently in use. There are also reports of poor quality or sometimes fake antimalarial drugs. When quality antimalarial drugs are available, the cost may be prohibitive for many people in developing countries. Thus, many individuals who live in endemic areas have started to identify and use plant-derived antimalarial remedies in their local environments. The diversity of resistance types of the parasite require region-specific measures in the control of malaria [4]. Malaria is characterized by cycles of chills, fever, pain and swelling. Of the species of malarial parasite currently known today, the most pathogenic type is *Plasmodium falciparum* as it can be life threatening. The other three species of malarial parasites, *P. vivax*, *P. ovale* and *P. malariae* are generally less pathogenic and are not life threatening [5]. *P. falciparum* is the most dominant and pathogenic and is also responsible for almost all malaria-induced mortality in tropical and subtropical countries [6] where the temperature and rainfall are optimum for the development of vectors and parasites [7]. The organism that causes the most severe form of malaria, *P. falciparum*, has developed resistance to nearly all synthetically manufactured drugs used for malaria treatment.

Garcinia kola Heckel belongs to the family Guttiferae and is a large forest tree which is well valued in most parts of West and Central Africa for its edible nuts [8]. The seed, known as bitter kola or false kola, is commonly chewed and serves as an alternative to true kola nuts scientifically known as *Cola nitida* and *Cola accuminata.* Bioactive extracts obtained from different parts of the plant and the nuts are used extensively in traditional African medicine, especially for the preparation of remedies for the treatment of laryngitis, cough and liver diseases among others [9, 10].

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This study was therefore performed to test the *in vivo* antimalarial activity of *Garcinia cola* in order to ascertain if there is any scientific justification for the use of *Garcinia kola* as a remedy for malaria.

2. Materials and Methods

2.1. Sample collection, preparation and extraction

Garcinia kola seed was purchased from Ogige market, Nsukka, Enugu State, Nigeria. The samples were identified by an experienced taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria. The samples were cut into small pieces and spread to dry at room temperature in the laboratory for about three weeks. They were then ground into fine powder and put in transparent polythene bags. The extraction procedure described in our previous studies was used in this study [11, 12]. Briefly, fifty grams of powdered seeds of the *Garcinia kola* were placed in 500 ml conical flask; ethanol was added until the samples were completely submerged in the solvent. The mixture was then agitated for thorough mixing and then put in a rotary shaker for 24 hr to ensure effective extraction of the plant components. The extract was filtered using Buchner funnel; Whatman number 1 filter paper and a vacuum pump. The solvent was evaporated using rotary vacuum evaporator (R -11) in a water bath at 40 °C. The extract was brought to dryness using vacuum pump at room temperature. The residue thus obtained was used for the experiment.

2.2. Preparation of plant extract and chloroquine sulfate stock solutions

The method described by Iwalokun [13] was used. Briefly, stock solutions of the plant extract were prepared by dissolving 0.5 g of plant extract in 5 ml of dimethylsulfoxide (DMSO) and obtaining a final dose of 400 mg/kg body weight. DMSO served as a negative control. A tablet of chloroquine phosphate (Evans Medical Co, Nigeria) was dissolved in distilled water to achieve a final dose of 10 mg/kg body weight of mice and served as the positive control. Each tablet of chloroquine sulfate contains chloroquine BP, 250 mg.

2.3. Parasite strain

Plasmodium berghei used in this research was obtained from University of Nigeria Veterinary Teaching Hospital, Nsukka, Nigeria. This strain of parasite was maintained by passaging the parasite in uninfected mice.

2.4. Experimental animals

Albino mice were obtained from the animal house in the Department of Veterinary Pathology, University of Nigeria Nsukka. The guidelines approved by the University of Nigeria Teaching Hospital, Enugu for the use of laboratory animals was followed for the study. Mice were given free access to feed and tap water.

2.5. Passaging of P. berghei parasite in mice

Standard inoculum was prepared from a donor mouse infected with *P. berghei* by adding 2 drops of blood from mouse in 0.8 ml of phosphate buffered saline (PBS). Infected blood from the donor mouse was obtained by snipping tip of the tail. Each mouse was infected with a standard inoculum of parasitized erythrocyte suspension in phosphate buffered saline (0.2 ml) [13].

2.6. Experimental design

Mice weighing 20 - 28 g were distributed into 5 groups of 3 mice each. In group 1, the mice were not infected with *P. berghei* and they were not given any treatment. This is the negative control group. In group 2, mice of known weight were used and the plant extract was given to the mice according to their body weights. This was repeated for five days before they were infected with *P. berghei*. The animals were treated with the dose of 400 mg/kg body weight plant extract until the experiment terminated. In group 3, mice were infected with the strain of *P. berghei* and this group served as the positive control. These mice were not treated and were allowed to die so as to discover how long it would take the parasite to kill each mouse. In group 4, the mice were infected with *P. berghei* and treated with chloroquine phosphate solution. In group 5, the mice were infected with *P. berghei* and treated with both the plant extract and chloroquine phosphate solution.

2.7. Thin film preparation

Thin blood films of experimental animals were prepared by placing a drop of blood at the edge of a clean glass slide (close to one end of the slide), and the edge of another slide (spreader) was inclined at an angle of 30° close to the drop of blood. The blood was spread to the edge of the spreader and then pulled forward slowly and steady. Then the film was allowed to dry, before staining.

2.8. Staining technique

The thin film was fixed with methanol solution, and allowed to dry for 1 minute thus preventing the film from washing off as previously described by Okeola at al. [14]. The thin films were then stained in a 10% solution of Giemsa stain prepared with a buffered water of pH 7.2 and allowed for 30 min, after which the slides were washed with clean water and the back of the slide was cleaned with cotton wool. The slide was placed vertically on the bench to dry before examining the films under the ×100 oil immersion microscope. The result was then scored as negative (if no parasite) or positive (if there is parasite) after examining the microscopic fields.

2.9. Determination of parasitaemia and parasite count

Parasitaemia was established by microscopic examination of a thin blood film under oil immersion ×100 magnification and measured as a percentage of infected erythrocytes in fields of 500 erythrocytes [13]. Percentage parasitaemia was counted using the formulae below.

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

2.10. Estimation of hemoglobin

Percentage of hemoglobin was measured to check if the mice were anemic, because the hemoglobin carries oxygen and is involved in several cellular functions. It needs to be in the normal range for mice to be healthy. Briefly, 2 ml syringe was used to collect mice blood. The blood was dispensed into a test tube and two drops of anticoagulant was added to it. The blood and the drabkin's solution was thoroughly mixed and allowed to stand for 5 min to allow for complete reaction. The drabkin's solution was then used as a blank in the colorimeter and the absorbance reading of test solution was noted at 540 nm. The result was obtained by using the formula below.

$$\text{Result} = \frac{\text{Absorbance of test} \times \text{Concentration}}{\text{Absorbance of standard}}$$

2.11. Estimation of the white blood cell count (WBC)

White blood cell count (WBC) is a test mostly carried out to note how healthy or infected a patient is. However, certain conditions are capable of producing a variation in the white blood cells e.g. leukocytosis is a term use to describe a condition where there is increased white blood cells more than 11,000 per cubic mm, while leucopenia is a decrease in same cells below 4,000 per cubic mm. Briefly, a 1 in 20 dilution of the sample was made by adding 20 µl of anticoagulated blood sample to 0.38 ml of Turk's solution. It was mixed and allowed to stay for 5 minutes. A drop of the mixture was added to Neubauer counting chamber and allowed to stay for 2 min for the cells to settle on the slide. The WBC was counted using ×10 objective of the microscope. The cells were counted in 4 large corner squares of the chamber marked W1, W2, W3 and W4. The cells lying on the lines of two sides of each large square were included in the count. The number of white cells per liter of blood (WBC) was obtained by first dividing the total

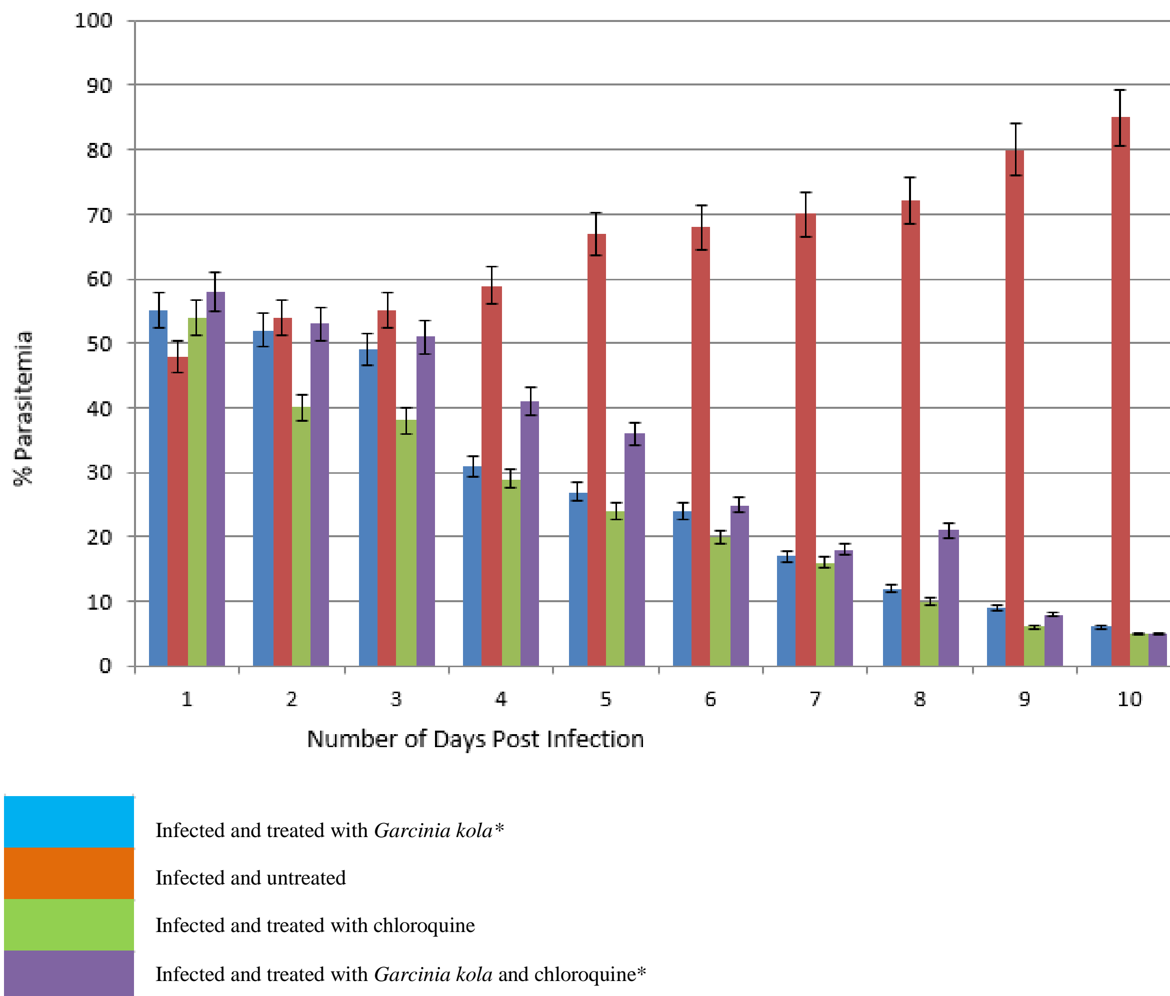

Figure 1. Percentage parasitaemia of mice infected with *Plasmodium berghei* and treated with *Garcinia kola*, infected and treated with chloroquine, infected and untreated, infected and treated with *Garcinia kola* and chloroquine. Data is given as mean ± SEM; one-way analysis of variance followed by post hoc test; *$p < 0.05$.

number of cells counted by 2 and dividing the number obtained by 10 and then multiplying by 10^9.

2.12. Estimation of the packed cell volume (PCV)

This was used to check the level of red cell in the blood to ascertain if anemia was present. Briefly, the blood was mixed properly, the capillary tube was put in the container and it was taped with the finger. The force that is being exerted, that makes the blood to rise in the capillary tube is called capillary force. The capillary tube is filled up to 2/3 and thereafter, sealed with the sealant and put to spin in the centrifuge for about 5 min. The reading was then obtained with the aid of a hematocrit reader.

2.13. Statistical analysis

Results were analyzed using SPSS version 20 for windows. Comparisons were made between negative control, positive control and treatment groups of various doses using one-way analysis of variance (ANOVA). Values of p less than 0.05 were considered significant.

3. Results

The effect of *Garcinia kola* on the infected mice with *Plasmodium berghei* is shown in Figure 1.The result showed that there was an increase in percentage parasitaemia in infected mice from day 1 up until day 2 post infection. A decline in percentage parasitaemia was observed from day 3 to day 10. On day 10, it reduced to 6 percent. Results of percentage parasitaemia of mice infected with *Plasmodium berghei* and untreated (positive control) showed that there was a progressive increase in percentage parasitaemia day 1 of post infection in the untreated group. The increase in percentage parasitaemia continued up until the last mouse in this group died. This observation clearly shows the antimalarial effect of *Garcinia kola* extract in the group of mice treated with this plant extract ($p = 0.023$), and also in the group treated with *Garcinia kola* extract and chloroquine sulfate combination ($p = 0.039$) when compared with infected and untreated groups respectively. Malaria parasites were found in the blood of the infected mice on the first day confirming that the animals were successfully infected.

A decline in percentage parasitaemia was observed from day 2 to day 10 when parasitaemia was 6 percent. Results of percentage parasitaemia of mice infected with *Plasmodium berghei* and treated with chloroquine showed that there was an increase in percentage parasitaemia in infected mice from day 1 until day 2 post infection. There was an observed decline in percentage parasitaemia from day 3 all the way to day 10. Subsequently, parasitaemia decreased to 6 percent.

The result of the experiments on hematological parameters is shown in Table 1. The data showed that the mice that were not infected and treated, had normal range of packed cell volume, white blood cell, red blood cell, hemoglobin and platelet counts. This shows that the mice in this group are healthy and not anemic. Those that were infected and treated with *Garcinia kola* extract were healthy and not anemic too. There was a decrease in the packed cell volume and hemoglobin of those that were infected but not treated indicating that the mice in this group were not healthy and were anemic possibly due to the presence of the *Plasmodium berghei* ($p = 0.042$). Mice that were infected and treated with chloroquine and *Garcinia kola* were healthy and not anemic. Finally, mice that were infected and treated with chloroquine were also healthy and not anemic.

Table 1. Result of hematological parameters

Groups	PCV (%)	WBC ($\times 10mm^3$)	RBC ($\times 10^6$)	Hb (g/dl)	Platelets ($\times 10mm^3$)
1	42.0 ± 0.31	11233.3 ± 0.72	10.9 ± 0.15	14.13 ± 0.14	5066.7 ± 0.47
2	39.7 ± 0.33	10766.7 ± 0.32	10.5 ± 0.40	13.8 ± 0.11	4833.3 ± 0.41
3	23.0 ± 0.35	9033.3 ± 0.17	5.3 ± 0.34	8.30 ± 1.40	4333.3 ± 2.13
4	30.7 ± 0.44	10633.3 ± 0.31	9.7 ± 2.22	11.1 ± 0.01	5200.0 ± 0.93
5	35.0 ± 0.12	10866.6 ± 0.15	9.5 ± 0.06	11.0 ± 0.01	4433.3 ± 0.52

Normal ranges of hematological parameters tested: PCV = 35-45; WBC = 9000-16000; RBC = 10.00-10.400, Hb= 8.0 -14.0, Platelets = 9000-25000. Group1 (were not infected with *Plasmodium* and were not given treatment), Group 2 (infected with *Plasmodium* and were treated with *Garcinia kola* extract), Group 3 (infected with *Plasmodium* and were not treated), Group 4 (infected with *Plasmodium* and were treated with chloroquine), Group 5 (infected with *Plasmodium* and were treated with both chloroquine and *Garcinia kola* extract). Each value represents the mean ± standard error of mean.

4. Discussion

The increasing failure of many antimalarial drugs in many malaria endemic regions has generated tremendous public health concern and obviously ignited interest in the exploration of alternative treatment options including medicinal plants and herbs as new antimalarial agents. Nature has been a great source of medicine for thousands of years, and an impressive number of new drugs have been isolated from natural sources, many of which are based on their use in traditional medicine. Various plants with therapeutic properties have been used for years by humans in the course of their daily lives. Bioactive plant compounds have served as templates for several synthetic drugs and as precursors for the production of semi-synthetic drugs.

In the present study, the *in vivo* antimalarial activity of *Garcinia kola* extract was evident. This finding is supported by data from other studies by different authors [15, 16]. The administration of *Garcinia kola* in mice prior to infection with *Plasmodium berghei* significantly reduced the percentage of parasitaemia on day of 3 post infection period compared to percentage parasitaemia on day 3 of post infection period in untreated mice. Reduction in parasitemia

was also observed in mice fed with *Garcinia kola* extract and additionally treated with chloroquine. In this later group, 5 percent parasitaemia was observed in a shorter time period i.e. day 10 of post infection period while in the group treated with *Garcinia kola* extract, 6 percent parasitaemia was attained on day 10 of post infection period. Similar findings were reported by Iwalokun [13] on enhancement of antimalarial effects of chloroquine using *Vernonia amygdalina* leaf extracts in mice infected with chloroquine-resistant and chloroquine-sensitive *Plasmodium berghei* strains. We had also previously showed in a different study that medicinal plant extracts can synergistically act with modern antibiotics *in vitro* [17]. This is a good development especially in an *in vivo* model such as the one used in the current study.

We noted that as the number of days increased in the post infection period, percentage suppression increased except in positive and negative controls. In the positive control group, there was a steady increase in percentage parasitaemia and no suppression was observed. However, the antimalarial component of this seed could have been responsible for the survival of the mice treated with *Garcinia kola* extract throughout the period of the experiment, when compared to the positive control group that died before the experiment was terminated. Interestingly, there are reports indicating that *Garcinia kola* has antimicrobial activity and some authors have attributed the anti-plasmodium activity of *Garcinia kola* to the presence of bioflavonoids [15, 16].

In conclusion, the results of this study justify the antimalarial property of *Garcinia kola* extract. Therefore, further studies should be aimed at testing more *Plasmodium* strains in addition to establishing the safety profile of this plant extract.

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