Original Research Article

Ethanolic Extract of *Garcinia kola* (Heckel) Seed possesses Antiplasmodial Properties against *Plasmodium berghei*

ABSTRACT

Background: *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 parasites have developed resistant to orthodox drugs over the years, thus need for herbal remedy*.

Aim: *This study therefore sought to investigate the* antiplasmodial properties of ethanolic extract of Garcinia kola seed against *Plasmodium berghei* in infected Swiss albino mice.

Methods: *G. kola* seeds were purchased from a local market in Ibadan, Nigeria. The seeds were chopped to smaller pieces after the outer coats were removed. They were air-dried and finally ground to fine powder using a blender. The powder was extracted using ethanol as the solvent. Sixty Swiss albino mice weighing obtained from the Federal University of Agriculture, Abeokuta, were acclimatized for 7 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 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 *G. kola* seed extract respectively. All treatments were orally done twelve hourly for five consecutive days from when parasites were first seen in the infected animal blood. Parasitaemia count and PCV were done using standard methods.

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

Conclusion: The result of this study showed that ethanolic extract of *G. kola* seed possesses antiplasmodial properties against *P. berghei* in a dose-dependent manner. Maximum antimalarial potency of plant extracts and standard antimalarial drugs can be derived when dosage is completed.

Keywords: Antiplasmodial properties, *Garcinia kola, Plasmodium berghei*, Swiss albino mice

1. INTRODUCTION

Garcinia kola (Heckel) is forest tree indigenous to sub-Saharan Africa and has been referred to as a 'wonder plant' because almost every part of it has been found to be of medicinal importance [1]. It occurs naturally from Sierra Lone to Southern Nigeria and on into Zaire and Angola, but is further distributed by man and is often found cultivated around villages. *G. kola* belongs to a family of tropical plants known as Guttifera [2]. It is an evergreen tree grown in the tropical rainforest of West Africa [3,4]. It grows to a height of about 30metres high, and the fruit, which is in the size of an orange, is smooth and reddish yellow with peach-like skin and yellow pulp and contains three or four seeds covered with brown seed coat [5]. The seed is an edible nut [4]. It is generally known and called Bitter Kola in Nigeria, and commonly called "Namiji

goro" in Hausa, "Orogbo" in Yoruba and "Aku-ilu" in Igbo [6].

Fig. 1: *Garcinia kola* Seeds [7]

The seed is a masticatory used in traditional medicine, cultural and social ceremonies. Extractive of the plant have been traditionally used for ailments such as laryngitis, liver diseases and cough [8]. The seeds are used to prevent or relieve colic, cure head or chest colds and relieve cough [9]. The seed also has anti-inflammatory, antimicrobial, antidiabetic and antiviral [10] as well as antiulcer properties [11]. Airaodion et al. [7] reported its hepatoprotective potency against acute ethanol-induced oxidative stress.

Phytochemical and biochemical studies of *G. kola* showed the presence of sterols, terpenoids, flavonoids, glycosides, pseudotannins, saponin, proteins and starch [12,13]. Maduniyi [14] reported that some workers isolated kolanone, a poly-isoprenyl-benzophenone compound from the fruit pulp. *G. kola* is a reasonable source of ascorbic acid, some micro-elements including nitrogen, potassium, phosphorus, magnesium and calcium, a trace amount of chromium [15]. Another medicinal constituent of *G. kola* is hydroxycitric acid (HCA) [16]. Xanthones, xanthone derivatives, and polyisoprenylated benzophenones have also been isolated from *G. kola* [17,18].

G. kola also contains toxic substances such as tannins, phytic and hydrocyanic acids at a low concentration. Other constituents include ash and crude protein, crude fiber, crude lipid, water–soluble oxalate, terpenoids and fat [5].

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 [19]. 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 [19]. 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 [19].

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 [20]. 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 [21]. 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 [22]. This present study therefore, sought to investigate the antiplasmodial properties of ethanolic extract of *G. kola* against *P. berghei* in infected Swiss albino mice.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

G. kola seeds were purchased from a local market in Ibadan, Nigeria. The seeds were chopped to smaller pieces after the outer coats were removed. They were air-dried and finally ground to fine powder using a blender. 500 g of the powder was transferred to an 80% ethanol solution in a 1 litre round-bottomed flask, and kept airtight for 72 hours. It was filtered and the filtrate was concentrated by means of a rotary evaporator at 40 ^{o}C. The resulting residue was further air-dried. The percentage yield was 14.72% of the dried sample.

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

2.3 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 in literature were adopted [23,24]. 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 seed 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.

2.4 Parasitaemia Count

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 [23,24].

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 [19].

2.6 Statistical Analysis

Data were subjected to analysis using Microsoft Excel.

3. RESULTS

The result of body weight change, PCV and parasitaemia counts are presented in figs. 2-4 respectively.

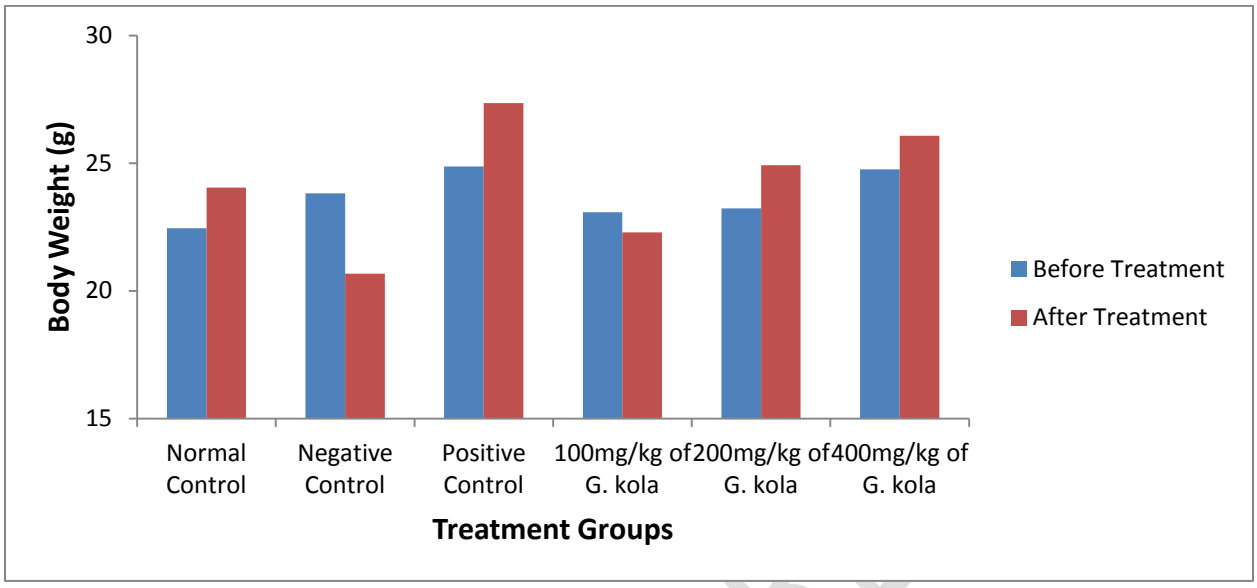

Fig. 2: Effect of Ethanolic Seed Extract of *G. kola* on body Weight of *P. berghei*-infected mice.

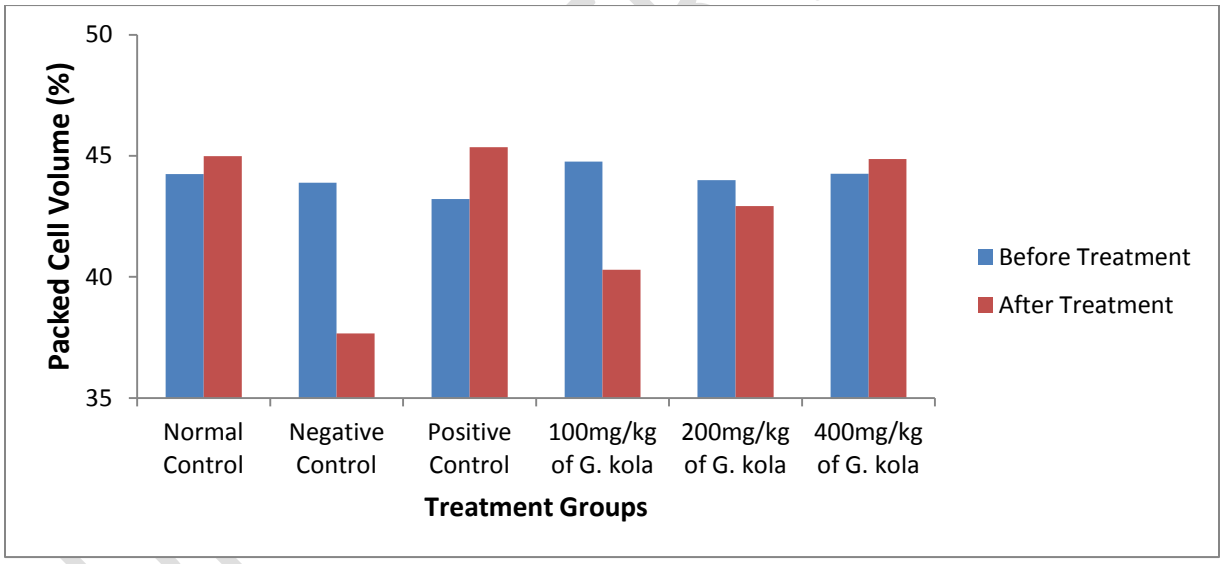

Fig. 3: Effect of Ethanolic Seed Extract of *G. kola* on Packed Cell Volume of *P. berghei*-infected mice

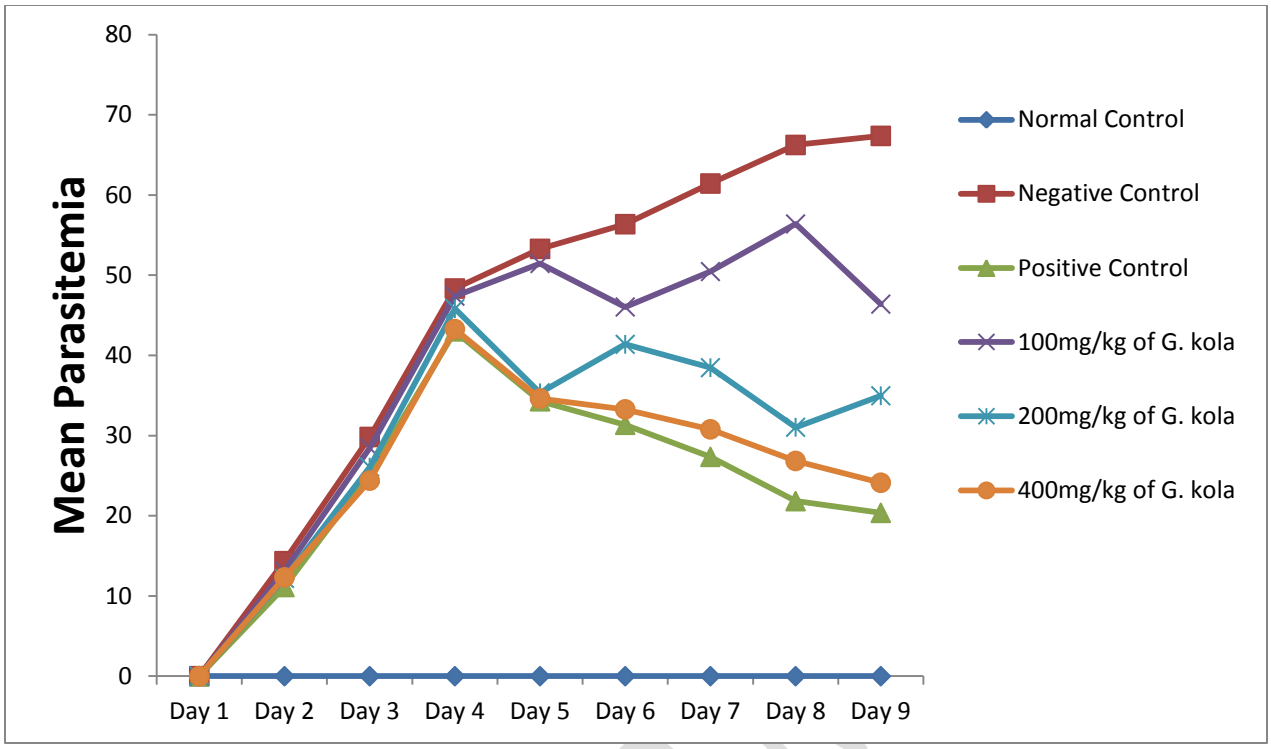

Fig. 4: *In vivo* Antiplasmodial Activity of Ethanolic Seed Extract of *G. kola* against *Plasmodium berghei* in infected mice*:* Each point represents Mean with n=10

4. DISCUSSION

Malaria is still considered a major public health problem in developing countries [19]. The malaria parasite has develop resistant to orthodox drugs over the years, thus need for herbal remedy. This present study therefore, sought to investigate the antiplasmodial properties of ethanolic extract of *G. kola* against *P. berghei* in infected Swiss albino mice.

From this study, the body weight of the infected untreated mice (negative control) showed significant weight lost after 4 days. The group treated with 100 mg/kg of *G. kola* seed has a slight reduction in their body weight after 4 days treatment (fig. 2). Interestingly, the groups treated with 200 mg/kg, 400 mg/kg of *G. kola* seed showed weight gain after 4 days of treatment. This might be an indication that the extract has the propensity to combact weight loss at these doses. Weight gain was also observed in the group treated with 5 mg/kg chloroquine (positive control). This is similar to the result of Airaodion et al. [19, 25] who treated *Plasmodium berghei*-infected mice with ethanolic leaf extracts of *Carica papaya* and *Vernonia amygdalina* respectively. Body weight loss and fever are some features of malaria infection [26]. Therefore, a potent antimalarial plant should be able to prevent body weight loss in infected mice [27]. In the present study, extract of *G. kola* was observed to have prevented body weight loss linked with elevation in parasitaemia level.

Anaemia has also been reported as one of the features of malarial infection in mice [18]. Anaemic condition results from haemolysis [28,29]. In this study, the effect of *G. kola* seed on packed cell volume (PCV) of *P. berghei*-infected mice is shown in Figure 3. The PCV of *P. berghei* infected untreated mice (negative control) and infected treated with 100 mg/kg of *G. kola* showed significant decrease in PCV

after 4 days of treatment. This shows that *P. berghei* infection significantly reduced red blood cells of animals and treatment with 100 mg/kg of *G. kola* was unable to remedy this situation. However, treatment with 200 mg/kg, 400 mg/kg of *G. kola* seed extract as well as those treated with 5 mg/kg chloroquine (positive control) showed significant increase in PCV after 4 days of treatment. The increase in PCV and body weight in mice treated with *G. kola at* 200 and 400 mg/kg when compared with the negative control group is suggestive of the ameliorating potency of *G. kola* seed extract on the anaemia induced by the *P. berghei* infection.

Parasitaemia level is the major parameter in determining malarial infection. In this study, the mean parasitaemia level of the *P. berghei* in infected mice treated with ethanolic seed extract of *G. kola* is shown in Figure 4. No noticeable difference was observed in the parasitaemia level of all the infected mice after 3 days of treatment. However, the difference became noticeable after 4 days of treatment. This might be suggestive that using this extract for 3 days might not yield significant antimalarial potency. It could also mean that usage of antimalarial drugs prescribed for 4 days might not yield maximum result if the dosage is not completed.

The result of this study also indicate that extract of *G. kola* seed 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.

G. kola seeds has been reported to be rich in phytochemical composition [12,13]. Flavonoids have been reported to have exhibited significant *in vitro* antimalarial activity against *P. falciparum* [25,30]. This could justify the antimalarial activities exhibited by the plant extract.

4. CONCLUSION

The result of this study showed that ethanolic extract of *G. kola* seed possesses antiplasmodial properties against *P. berghei* in a dose-dependent manner. Maximum antimalarial potency of plant extracts and standard antimalarial drugs can be derived when dosage is completed.

5. ETHICAL APPROVAL

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

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