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 resistance to orthodox drugs over the years, thus the 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 air-dried and ground to fine powder using a blender. The powder was extracted using ethanol as the solvent. Sixty Swiss albino mice were 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 packed cell volume 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,* malaria, *Plasmodium berghei*

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 is of medicinal importance [1]. *Garcinia kola* belongs to a family of tropical plants known as Clusiaceae [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]. 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 [31].

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

The seed is a masticatory used in traditional medicine, cultural and social ceremonies. Extractive of the plant has 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 fibre, crude lipid, water-soluble oxalate, terpenoids and fat [5].

The antimalarial potential of compounds derived from plants is proven by examples such as quinine, obtained from *Cinchona* species, and artemisinin derivatives, obtained from *Artemisia annua* [19]. The selection of plants to be screened for antimalarial activity is done based on 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 that have antiplasmodial properties [19].

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]. 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 [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. falciparum* malaria has led to the wide-spread appearance of CQ-resistant strains against *P. falciparum* 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 a 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 using a rotary evaporator at 40 ^{o}C. The resulting residue was further air-dried. The percentage yield of the dried sample was calculated.

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 Swiss albino mice weighing 20 - 25 g were obtained from the Animal House of Federal University of Agriculture, Abeokuta, Nigeria. They were acclimatized for seven 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. To evaluate the curative potential of the crude extract, the methods described in the 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 methanol and stained with 10% 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 was calculated 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 centrifuged for 5-6 minutes. The packed cell volume was read directly from haematocrit reader [19].

2.6 Statistical Analysis

Bars were plotted using Microsoft Excel. Statistical analysis was done using Graph Pad Prism version 5. One way analysis of variance (ANOVA) was used to compare means and data were considered significantly different at 95% confidence level ($P<0.05$).

3. RESULTS

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

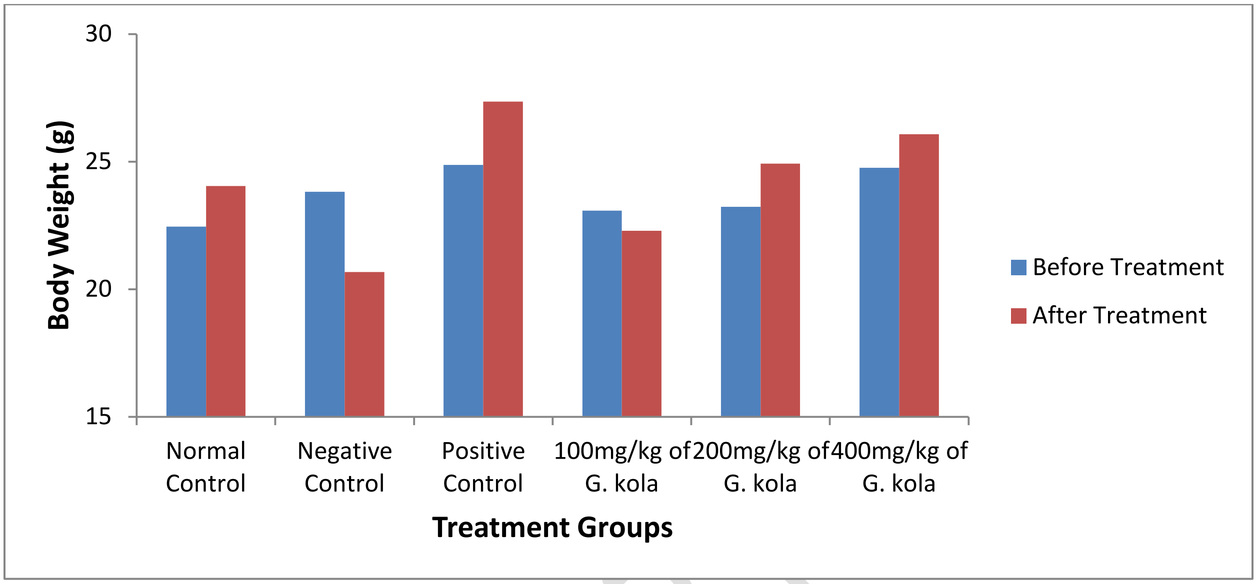

Fig. 2: Effect of Ethanolic Seed Extract of *G. kola* on body Weight of *P. berghei-i*nfected mice.

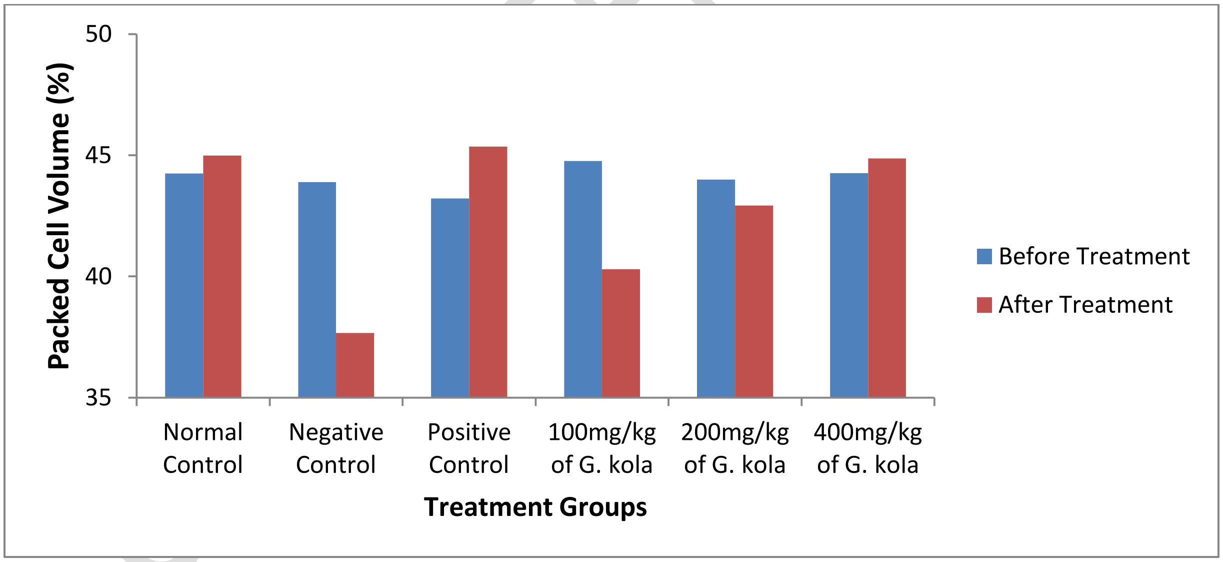

Fig. 3: Effect of Ethanolic Seed Extract of *G. kola* on Packed Cell Volume of *P. berghei*-infected mice

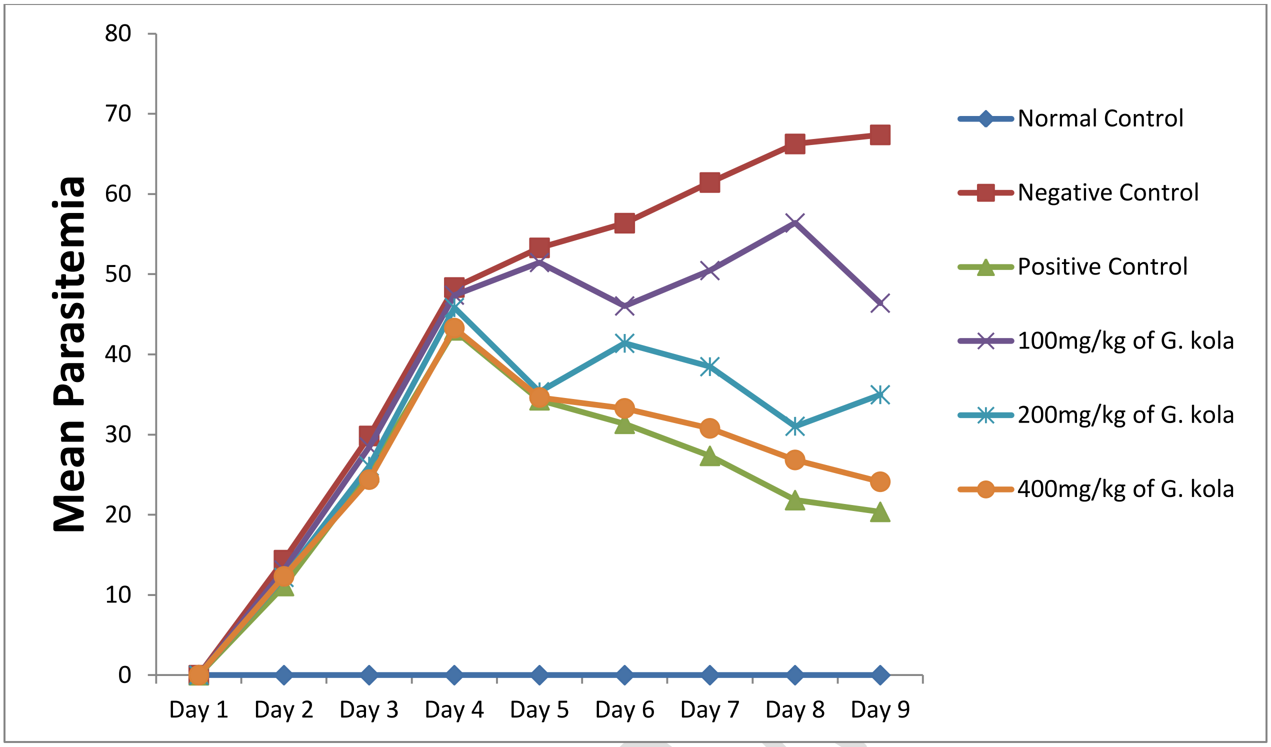

Fig. 4: *In vivo* Antiplasmodial Activity of Ethanolic Seed Extract of *G. kola* against *Plasmodium berghei* in infected mice*:* Each point represents Mean with n=10

4. DISCUSSION

Malaria is still considered a major public health problem in developing countries [19]. The malaria parasite has developed 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 bodyweight of the infected untreated mice (negative control) showed significant weight loss after 4 days. The group treated with 100 mg/kg of *G. kola* seed had 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 combat 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. Bodyweight 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, an 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]. The 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 a 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 a 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 the usage of antimalarial drugs prescribed for 4 days might not yield the maximum result if the dosage is not completed.

The result of this study also indicates 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 favourably with chloroquine, the standard antimalarial drug used in this study.

G. kola seeds have 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 the 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 was written ethical approval has been collected and preserved by the authors.

6. REFERENCES

1. Hutchson J, Dalziel JM. Flora of West Tropical Africa.2nd Ed. H.M.S.O London. 1956;8(11): 295.
2. Plowden CC. A manual of plants names. 3rd ed. London. George Ltd: 1972; p239.
3. Burkhill HM. The useful plants of west tropical Africa vlume 2.Assessed from http://www.aluka.org – a digital library of scholarly resources from and about Africa. On 10/11/2011.
4. Ofusori DA. Ayoka AO, Adelekun AE, Falana BA, Adeeyo OA, Ajeigbe KO, Yusuf UA. Microanatomical Effects of Ethanolic Extract of Garcinia Kola on the Lungs of Swiss Albino Mice. *The Internet Journal of Pulmonary Medicine*. 2008;10:1
5. Aniche GN, Uwakwe GU. “Potential use of *Garcinia kola* as a hop substitute in lager beer brewing”.*World Journal of Microbiology and Biotechnology.* 1990;(6): 323 – 486.
6. Bnouham M, Abderrahim Z, Hassane M, Abdelhafid T, Abdelkhaleq L. "Medicinal plants with potential antidiabetic activity – A review of ten years of herbal medicine research (1990-2000)". *International journal of Diabetes & Metabolism* 2006; 12(14):1–25.
7. Airaodion AI, Ngwogu AC, Ekenjoku JA, Ngwogu KO. Hepatoprotective potency of ethanolic extract of *Garcinia kola* (Heckel) seed against acute ethanol-induced oxidative stress in Wistar rats. *International Research Journal of Gastroenterology and Hepatology*. 2020;3(2):1-10.

8. Anyensu ES. Medical Plants of West Africa.*Reference Publication Incoporated*; Algonac, MI: 1978; p162.
9. Iwu MM. Pharmacognotical Profile of Selected Medicinal plants. In: Handbook of African Medicinal plants. CRC press, Boca Raton, Florida: 1993; p183.
10. Iwu MM. In plant flavonoids in Biology and Medicine. V. Cody, E. Middleton and J.B Harbone eds. Ala R. Liss. New York: 1986; p485.
11. Ibironke GF, Olaleye SB, Balogun O, Aremu DA. Antiucerogenic effect of diets containing seeds of *Garcinia kola* (Hekel) *Phytotherapy Research.* 1997; 4(11):312-313.
12. Igboko AO. Phytochemical Studies in *Garcinia kola*." Hekel. Msc. Thesis, University of Nigeria Nsukka, Nigeria, (Unpublished). 1983.
13. Braide VD, Vittrotio G. Histological Alterations by a Diet Containing Seeds of *Garcinia kola*: Effects on liver, Kidney and intestine in the Rat. In*: Gedenbaurs Morphology. Jahrb, Leipzig*. 1989;1334 I.S. 95-101.
14. Maduinyi, I. Biochemical and pharmiocol studies of the active principles of the seeds of *Garcinia kola*". M.Sc Thesis, University of Nigeria Nsukka, Nigeria. (unpublished). 1983.
15. Eka OU. Studies in the feasibility of replacing hop by other bittering substances in brewing. *Nigerian Journal of Microbial.* 1984;4(1-2):43-51
16. Jena BS, Jayaprakasha GK, Singh RP, Sakariah KK. Chemistry and biochemistry of hydroxycitric acid from *Garcinia*. *Journal of Agriculture, Food and Chemistry*. 2002;50(1):10-22.
17. Masullo M, Bassarello C, Suzuki H, Pizza C, Piacente S. Polyisoprenylated benzophenones and an unusual polyisoprenylated tetracyclic xanthone from the fruits of Garcinia cambogia.*Journal of Agriculture, Food and Chemical Toxicology*; 2008;56(13):5205-5210.
18. Koshy AS, Anila L, Vijayalakshmi NR. Flavonoids from *Garcinia cambogia* lower lipid levels in hypercholesterolemic rats. *Food and Chemical Toxicology*. 2001;72(3):289-294.
19. Airaodion AI, Airaodion EO,Ekenjoku JA, Ogbuagu EO, Ogbuagu U. Antiplasmodial Potency of Ethanolic Leaf Extract of *Carica papaya* against *Plasmodium berghei*in Infected Swiss Albino Mice. *Asian Journal of Medical Principles and Clinical Practice.* 2019;2(2):1-8
20. Miller LH, Good MF, Milon G. Malaria pathogenesis. Science 1994; 264: 1878-1994.
21. WHO. The world health report-changing history. Geneva: World Health Organization; 2004, p. 96.
22. Peters W. Antimalarial drug resistance: an increasing problem. Br Med Bull 1982; 32: 187-192.
23. Akuodor GC, Idris UI. Anti-nociceptive, anti-inflammatory and antipyretic effect of the methanolic extract of *Bombax buonopozense* seeds in rats and mice. *African Journal of Biotechnology*. 2011; 10:3191-3196.
24. Ryley J, Peters W. The antimalarial activity of some quinoline esters. *Ann Trop Med Parasitology.* 1995;84(22):209–2.
25. Airaodion AI, Airaodion EO, Ogbuagu U, Ekenjoku JA, Ogbuagu EO. Antimalarial efficacy of ethanolic seed extract of Vernonia amygdalina against *Plasmodium berghei* in infected Swiss albino mice. *International Journal of Bio-Science and Bio-Technology*. 2019;11(8):68-76.
26. Langhorne J, Quin SJ, Sanni LA. Mouse models of blood-stage malaria infections: Immune responses and cytokines involved in protection and pathology. In: Perlmann P, Troye-Blomberg M, editor. *Malaria Immunology. Stockholm: Karger Publisher.* 2002;204–228.
27. Bantie L, Assefa S, Teklehaimanot T, Engidawork E. *In vivo* antimalarial activity of the crude seed extract and solvent fractions of *Croton macrostachyus* Hocsht. (Euphorbiaceae) against *Plasmodium berghei* in mice. *BMC Complement Altern Med*. 2014;14:79.

28. Airaodion AI, Ogbuagu U, Ekenjoku JA, Ogbuagu EO. Comparative Assessment of Haematopoietic Potential of Ethanolic Extract of *Telfairia occidentalis* and *Talinum triangulare* Leaves in Wistar Rats. Acta Scientific Nutritional Health. 2019;3(10):38-43.
29. Airaodion AI, Ogbuagu EO, Ekenjoku JA, Ogbuagu U, Airaodion EO. Haematopoietic Potential of Ethanolic Leaf extract of *Talinum triangulare* in Wistar Rats. *Asian Journal of Research in Biochemistry*. 2019; 5(2):1-7.
30. Chanphen R, Thebtaranonth Y, Wanauppathamkul S, Yuthavong Y. Antimalarial principles from *Artemisia indica*. Journal of Natural Products. 1998;61:1146-1147.
31. Damian, D. C., Nweze, E. I., & Onyeke, C. C. (2017). The in vivo anti-plasmodium activity of Garcinia kola Heckel. *Journal of Basic Pharmacology and Toxicology*, *1*(2), 27-31.