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Antimalarial activity of Garcinia Kola in Abino Mice

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Garcinia kola is a member of the Clusiaceae family, which is used in African traditional medicine. *G. kola* has antidiabetic, hepatoprotective, antiplasmodial, and hypolipidaemic properties. The research, therefore, evaluated the acute toxicity, phytochemical, antimalarial, and haematological parameters of the plant with a view to determining the haemato-pharmacological potential. The extraction of *G. kola* was carried out using methanol; it was filtered, evaporated to dryness, and tested for phytochemical constituents. Fifty (50) male and female mice were used, 25 for the suppressive, and another 25 for a curative model of antimalarial and haematological activities. The acute toxicity test (LD_{50}) was determined within 24 h. The suppressive model used Peter's 4-day protocol involving the administration of *G. kola* extract to three (3) groups of three mice *per* group, with doses of 100, 200, and 400 mg/kg, p.o. respectively of the extract, and 4 mg/kg, p.o. aqueous solution of Artemether/ Lumefantrine (AL) (80/480 mg), and 10 mL/kg, p.o. of distilled water to the 4th and 5th groups, for 3-days, after 4 h of inducing parasitaemia in the animals. The curative model used the Rane's protocol and involved a 3-day establishment of parasitaemia in mice (n=6) *per* group. Suppression of parasitaemia in the curative model was found to be 2.40±24.00, 1.20±38.00, 0.60±24.00, 0.40±.24.00, 34.40±2.29 *per* 10 fields of 1000 erythrocytes for the 100, 200, 400 mg/kg, p.o. of the *G. kola,* and 1 mg/kg, p.o. of AL against 28.00 *per* 10 fields of 1000 erythrocytes of the negative control, which was significant (P<0.05) both within the groups and the negative control. The haematological parameters (Hb, PVC, and RBC) showed significant (P<0.05) increases in their respective values. The result of the curative model demonstrated a daily (for 3-consecutive days) increase in parasitaemia clearance, an indication of antiplasmodial activity. The study concluded that *G. kola* has antimalarial activity with improvement in the red blood cell indices in albino mice.

Keywords: Acute Toxicity, Plasmodium, Arthemeter/ Lumefantrine, Hematological, Curative, Suppressive

INTRODUCTION

Malaria is a serious and potentially fatal multi-systemic disease of humans and animals, which is transmitted by the bite of infected female Anopheles mosquito (Feachem,2018) that carries the plasmodium parasite. Five parasite species cause malaria in humans; two of these – *P. falciparium* and *P. vivax* pose the greatest threat. A lot of strategies has been put in place to control the scourge, including vector control, preventive chemotherapy, use of malaria vaccine, etc. (Bass et al.2008) Rollback malaria partnership reports that malaria is epidemic in 117 countries with some 3.2 billion people living in the risk areas. They went further to state that *P. falciparum*, which is the parasite affecting humans has 74 % cases in Africa, 25 % in Asia, and 1 % in the Americas. The estimated number of malaria deaths stood at 627,000 in the year 2020 (Ugbaja et al.2017) with an estimated 241 million cases worldwide. Infection with the parasite affects blood cells and symptom of malaria is non-specific and resembles those of minor systemic viral illness. They comprise headache, lassitude, tiredness, abdominal discomfort, and muscle or joint aches, usually followed by increased temperature, chills, perspiration, loss of appetite, vomiting, and worsening malaise, which may progress to potential lethal severe malaria. Severe malaria usually manifests with one or more of the following: coma, acidosis, severe anemias,

hypoglycaemia, acute renal failure or acute pulmonary oedema. If it is left untreated, death may ensue (Sofowora et al.2013). The increasing failure of many antimalarial medications in many malaria-endemic regions has generated tremendous public health concern and rekindled interest in the exploration of alternative treatment options, including medicinal herbs and plants as new antimalarial agents. Herbal medicines go hand-in-hand with traditional African medicine, and also appears to be universal in different cultures, the varying factor being the mode of treatment and the plants used for the same ailments. Crude medicines of natural or biological origin have been isolated from plants that serve as templates or leads for several synthetic medications (Bulus et al.2011) Assessment of antimalarial efficacy and its effects on blood parameters, and weight variations of *G. kola* were the objectives of the research. The rationale for the analysis was to scientifically establish the benefits of *G. kola*, and develop it as a novel and potent anti-plasmodial agent.

MATERIALS AND METHODS

Study Area

The study was conducted at the Pharmacology and Toxicology Department, Animal Science sub-Laboratory, University of Nigeria, Nsukka from July, 2020 to September, 2021.

Chemical Reagents and Equipment

The materials and solvents used for the work, include powdered *Garcinia kola*, methanol (Methanex Co-operation, USA, 2019) N-hexane (Brentag Nigeria, 2017), Artemether/ Lumefantrine (AL) (80 mg/480 mg) (Artelum®, May & Baker, Nigeria), Ethyl acetate (New Arihant Chemicals, Mumbai, 2017), porcelain clothes, Whatman filter paper, refrigerator (Hajer Thermocool Plc., USA), glass beakers 500 L, kitchen knife, laboratory blender, measuring cylinder (glass wares), cages, syringes and needles, hand-gloves, hand towel, stop-watch, electron microscope (Speed Fair Company Ltd., Hong Kong, China), haematocytometer (counting chamber) (Kahan Care, Rohini, Delhi, India, 2018), red cell pipette, Ringers' solution, and cover-slip. Distilled water was procured from Energy and Resources Centre, University of Nigeria, Nsukka, as well as phosphate buffer. Other reagents were contained in the commercial kits, products of Randox, QCA, USA, biosystem reagents and instruments, Spain. The following instruments were used for the experiment: adjustable micropipette (Perfect, USA), chemical balance (Gallenkamp, England), digital photo-colorimeter (E1, 312 Model, Japan), incubator (Gallenkamp, England), microscope slides (Unescope, USA), pH meter (Pye, Unicam 293, England), refrigerator (Kelvinator, Germany), and spectrophotometer (Novaspec LKB Biochrome, Model No. 4049. All reagents were of analytical grade.

Plant Collection

Garcinia kola used in the study was collected from Orie-Orba market, near Nsukka in Enugu State, Southeastern Nigeria. The plant specimen and the seeds were identified and authenticated at the herbarium of the Pharmacognosy Department of the University of Nigeria, Nsukka by Mr. Felix Nwafor.

Preparation of Methanol Extract

The plant was sorted to remove any contaminants and then air-dried for 7-days. The dried plant seed was grounded into a fine powder with the aid of an electric dry mill (Moulinex). Ground powder of 1 kg was thoroughly soaked in 5L of methanol (Sigma-Aldrich, USA) and macerated 48 h at room temperature. At the expiration of time, the sample was sieved with porcelain cloth and further with Whatman filter paper. The filtrate was concentrated using a rotary evaporator at 45 ℃. The methanol extract was stored in a refrigerator at 4 ℃ till it was needed for use. Appropriate concentrations of the extract were subsequently made by serial dilution with distilled water.

Phytochemical Analysis

Phytochemical analysis tests were carried out on the extract at the Pharmacology & Toxicology Laboratory of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. Using standard procedures[6,7] preliminary phytochemical screening of the methanol extract of the plant was done. The extract was screened for the presence of tannins, saponins, glycosides, flavonoids, resins, anthraquinone, alkaloids, and other secondary metabolites.

Collection of *P. berghei*

The strain of *P. berghei* (NK-65 strain) *was* obtained from the Nigerian Institute of Medical Research, Lagos. The parasites were maintained through weekly blood passage from infected mice to uninfected mice kept in the laboratory.

Animals

A total of fifty mice weighing between 16 – 32 g were used for the study. The mice were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. The animals were fed with standard laboratory pellet diet and free drinkable water. They were housed in clean metal cages at room temperature. They were also maintained at a 12 h light/ dark cycle throughout the period of the experiment. They were allowed for one-week to acclimatise to the new environment before commencement of the study.

Ethics Approval
The study was approved by the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, and conducted in consonance with the guidelines for animal care in research (National Committee for Research Ethics in Science and Technology-Sass,2000).

EXPERIMENTAL DESIGNS
The study design involved three distinct experimental protocols. Firstly, the evaluation of blood schizonticidal activity in early infection (the 4-day suppressive test). Secondly, the determination of the curative antimalarial activity of the methanol extract of *Garcinia kola*. Thirdly, the haematological examination of the research plant. The animals used for the different models were drawn in three replicates from the integral group of animals for the specific drug/ extract and doses. For the curative, each group for 100, 200, and 400 mg/kg, p.o. for both aqueous and methanol extracts with the controls, has a total of five animals *per* group making a total of twenty-five (25). For the suppressive model, a total of twenty-five (25) mice were used having five animals (n=5) *per* group. From the five groups (100, 200, and 400 mg/kg, p.o. for methanol extract respectively, and negative and positive controls), replicate groups were drawn (n=5) *per* group for baseline and treatment analyses.

Acute Toxicity Test
The oral acute toxicity (LD_{50}) of *Garcinia kola* was determined in mice using the method previously described by Ozioma and Chinwe 2019. A total of 25 male and female mice were employed, in which acclimatisation period of 24 h was allowed with free access to drinkable water and food *ad libitum*. The extract was weighed and dissolved in dilute water. The test was carried out in groups of five animals (n=5) for each extract. Firstly, the extract was administered orally at doses of 100, 200, and 400 mg/kg, p.o. to each test group. The animals were monitored for 24 h and the number of deaths *per* group was recorded. Then, the mice were observed continuously for 1 h after treatment, intermittently for 4 h, and thereafter for 24 h. (Desye et al. 2020)

Antiplasmodial Assay

Paul's 4-Day Suppressive Test
Five groups of mice from the twenty (20) were inoculated with the parasite of *P. berghei* at the start of the experiment (Day 1). Methanol extract of *G. kola* was administered to three (3) groups of mice in graded doses of 100, 200 and 400 mg/kg, p.o. respectively, while group 4 (positive control received 4 mg/kg, p.o. of Artemether-Lumefantrine), and the 5th group, which served as a negative control, received 10 mL/kg, p.o. of distilled water. From the caudal vein of the animal, blood samples were collected (two drops) on the 4th day, and transferred to slides to make thin films from each mouse, stained with Leishman stains, with each stained slide examined under the microscope at 100 magnification power to determine the malarial percentage suppression of each extract group compared to control, thus finding out the average percent parasitaemia. In calculating the average percentage suppression of parasitemia in mice, this formula applies:

$$\text{Average (Av) \% Suppression} = \frac{\text{Av \% Parasitaemia in Control} - \text{Av \% Parasitaemia in Test}}{\text{Av \% Parasitaemia in Control}} \times 100$$

Rane's Curative Test
Twenty-five male and female mice were divided into three groups of five mice each, and each mouse was inoculated with the parasite on the 1st day of the experiment (Day 1). The mice were not treated until parasitaemia was established on the 4th day (i.e., 72 hours after the animals were infected). Groups 1, 2, and 3 of the mice received 100, 200, and 400 mg/kg, p.o. *per* day respectively of the methanol extract for 4-days, while group 4 was administered 4 mg/kg of Artemether-Lumefantrine, which served as the positive (standard) control. The 5th group was administered 10 mL/kg, p.o. of distilled water, which served as the negative control. On the fourth day (Day 4), two drops of blood were taken and transferred unto slides, thus making a thin-film from each mouse and staining with Leishman stain, so that the average percentage (%) parasitaemia can be calculated for each of the doses. After the fourth day, the animals were fed and observed. Deaths that occurred during this period were noted and used to determine the mean survival time. This method is a modification of Nardos and Makonnen,2017.

Haematological Assay

Collection of Blood Samples
A heparinised capillary tube was used to draw blood (through the retro-orbital route) from the mice, safely stored in EDTA bottles for determination of leucocyte count (differential), packed-cell volume, red blood cell count, and haemoglobin concentration, performed according to (Alaribe et al. 2020). The haematological analysis was carried out on the zero (0) day (baseline assay), and the fourth day.

Packed Cell Volume
Tilted at an angle of 30^0 and filled with blood was a haemostat capillary tube, sealed with plasticine at one end before centrifuging the whole process for 20 min at 300 rpm in a centrifuge. A haematocrit reader was used to read off the height of the packed-cell in percentages.

Haemoglobin Concentration
Determination of haemoglobin concentration was done using the Sahli haemoglobin metre 0.1 N HCL was the place to 10 mark of Sahli tube and 20 ul of blood was

placed into the tube with the Sahli blood pipette, and mixed up and down allowing it stand for 5 min to form acid haematin, which was diluted with distilled water. This slowed to a darker colour compared with that in the haemoglobin metre and the standard. Dilution continued until the colour got paler than the standard. Noted and taken were the volumes of the colour change, which did not exceed ±5 for the adoption of the average value.

Red Blood Cell Count

The method adopted was according to (Addai-Mensah et al.2019) using a dilution with a red mixer pipette sealed with the finger, blood was drawn up to 0.5 mark and Ringer's solution diluents drawn up to 101 mark, with shaking for proper mixing. Half of the content of the pipette was emptied into a waste container with a small amount of diluted blood and filled into a corner chamber of the haemocytometer. This was allowed for 1 min for the cells to settle, and then counted with x400 objective and focused with x100 objective.

Statistical Analysis

Statistical analysis was conducted using SPSS version 17.0 (SPSS Inc., USA). One-way analysis of variance (ANOVA) was used to test the differences between means groups. Dunnett test separated treatment means for statistical differences and compared a pair of group means. The level of statistical significance was set at 5 % (P<0.05).

RESULTS

Acute Toxicity

The LD_{50} of *G. kola* extract was ≥ 5000 mg/kg, p.o., and found to be safe in the test subjects.

Phytochemical Analysis

Phytochemical screening showed a heavy presence of carbohydrates and tannins, and moderate quantities of alkaloids, glycosides, saponins, flavonoids, proteins, steroids, and terpenoids. Present in little quantities are reducing sugar, resin, oil, and acidic compounds. The analysis demonstrated that balsam and phenols were absent in the methanol extract of the *G. kola.*

Table 1: Curative Effect of Methanol Extract of *G. kola* on Mice Infected with *P. berghe*

Treatment	Day 0	Day 1	Day 2	Day 3	Day 4
CTR	20.60±1.12	11.40±0.51*	7.80±0.73*	5.80±0.73*	2.40±0.24*
100 mg/kg	20.60±1.21	11.40±0.51*	7.80±0.73*	5.80±0.73*	2.40±0.24*
200 mg/kg	20.60±1.02	9.20±0.37*	6.20±0.37*	3.60±0.40*	1.20±0.38*
400 mg/kg	20.00±0.71	9.00±0.45*	4.60±0.24*	2.60±0.24*	0.60±0.24*
STD (A/L)	22.00±2.02	8.80±0.80*	5.80±0.37*	3.00±0.31*	0.40±0.24*

*The mean difference was significant at P<0.05. The result showed that there was a dose-dependent antiplasmodial activity of the extract with a consistent reduction in the plasmodium count.

Table 2: Pre- and Post Extract Administration Readings (Packed Cell Volume)

Treatment	PCV 0	PCV 1	PCV 2	Difference	% Difference
CTR	44.80±1.74	38.20±1.07	37.60±1.44	-0.60	-1.60
100 mg/kg	44.00±1.34	37.00±1.14	40.00±1.05	3.00	7.50
200 mg/kg	45.20±1.28	39.20±0.86	43.00±1.38*	3.80	8.80
400 mg/kg	43.20±1.02	37.40±0.75	42.80±0.86*	5.40	12.60
STD (A/L)	43.80±1.39	37.80±0.86	42.60±0.92*	4.80	11.30

*The mean difference was significant at P<0.05. There was a positive dose-dependent effect of the extract on PCV of the mice infected with plasmodium, showing an improvement in the packed cell volume.

Table 3: Pre- and Post Extract Administration Readings (Haemoglobin)

Treatment	Hb (0)	Hb (1)	Hb (2)	Difference	% Difference
CTR	13.72±0.22	14.54±0.22	13.72±0.22	0.50	3.60
100 mg/kg	14.42±0.26	13.50±0.14	13.50±0.14	0.42	3.00
200 mg/kg	14.72±0.15	13.68±0.17	13.68±0.17	0.46	3.20
400 mg/kg	14.52±0.15	13.76±0.13	13.76±0.13	0.54	3.80
STD (A/L)	13.72±0.15	14.56±0.17	13.72±0.15	-0.40	-

*The mean difference was significant at P<0.05. There was a positive dose-dependent effect of the extract on haemoglobin concentration, with an improvement in the haemoglobin levels.

Table 4: Effect of Methanol Extract of *G. kola* on Red Blood Cell Count

Treatment	RBC-0	RBC-1	RBC-2	% Difference
CTR	4.50±0.16	3.89±0.08	3.80±0.13	2.56
100 mg/kg	4.42±0.11	3.92±0.08	4.08±0.07	3.43
200 mg/kg	4.62±0.14	3.92±0.08	4.43±0.10*	11.51
400 mg/kg	4.29±0.04	3.89±0.08	4.29±0.03*	9.32
STD (A/L)	4.40±0.11	3.89±0.10	4.38±0.09*	11.19

*The mean difference was significant at $P<0.05$. The result showed a positive dose-dependent effect of *G. kola* extract on RBC of mice induced with *P. berghei*, with an improvement in red cell count.

Table 5: Effect of Methanol Extract of *G. kola* on Weight of Mice (Suppressive Model)

Treatment	Weight 1	Weight 2
CTR	19.83±0.76	21.42±1.11
100 mg/kg	28.31±1.40	28.71±1.24*
200 mg/kg	26.79±1.67	26.58±1.60
400 mg/kg	28.94±1.08	31.51±1.26*
STD (A/L)	25.41±1.41	28.32±1.79*

*The mean difference was significant at $P<0.05$. At weight 2, the weight of mice increased relative to control.

Table 6: Effect of Methanol Extract of *G. kola* on Mice Weight (Curative Model)

Treatment	Weight 1	Weight 2
CTR	30.32±2.75	29.66±3.00
100 mg/kg	18.60±0.61	19.60±0.51*
200 mg/kg	18.34±1.12	19.83±1.09*
400 mg/kg	18.51±0.81	19.87±0.89*
STD (A/L)	18.64±0.68	20.07±0.74*

*The mean difference was significant at $P<0.05$. The weights of mice significantly increased relative to control.

DISCUSSION

The work investigated *G. kola*'s *in vivo* antimalarial and haematological activities, as well as weight variations of its methanol extract in adult albino mice. The study was designed to determine the impact of the immune system on malaria eradication (Mahantayya et al.2016). *P. berghei* and at sometimes *P. vivax* can be utilised in an experimental model of malaria infection causing symptoms of pathology, which mimic symptoms produced by human malaria (e.g., *P. falsiparium*) Oluwatosin et al.2014 and J-West et al. 2020. This rodent malaria is dissimilar to the human plasmodium parasite, yet it is the starting point in screening most *in vivo* antimalarial activities of candidate compounds. Currently available antimalarial medications were discovered through this procedure, which involves preclinical (basic studies) and clinical investigations (human experimental trials). The effect of *G. kola* on the infected mice with *P. berghei* showed that there was an increase in the percentage parasitaemia in infected mice from day 1 up until day 2 post-infection. The decline in percentage parasitaemia was observed from day 3 to day 4. Results of percentage parasitaemia in mice infected with *P. berghei* and the untreated (positive control) showed that there was a progressive increase in the percentage parasitaemiaon day 1 post-infection in the untreated group. The increase in the percentage parasitaemia continued up until the last mouse in the group died. This observation clearly showed the antimalarial effect of *G. kola* extract in the group of mice treated with the plant extract, and also, in the group treated with *G. kola* extract and artemether-lumefantrine 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. The decline in the percentage parasitaemia was observed from day 2 to day 4. Results of percentage parasitaemia of mice infected with *P. berghei* and treated with artemether-lumefantrine indicated that there was an increase in the percentage parasitaemia in infected mice from day 1 until day 2 post-infection. There was an observed decline in the percentage parasitaemia from day 3 to day 4. Subsequently, parasitaemia decreased (tables 1 and 5). The results of the experiments on haematological parameters were shown in tables 3, 4, and 5, and figures 2, 3, and 4. The data showed that the mice that were not infected, but treated, had a normal range of packed cell volume, red blood cells, and haemoglobin. This showed that the mice in the group were healthy, but not anaemic. Those that were infected and treated with *G. kola* extract were healthy, but also, not anaemic. There was a decrease in the packed cell volume (Oyeyemi et al. 2019) and haemoglobin (Maourova et al.2019) of those that were infected, but not treated, indicating that the mice in the group were not healthy, but were anaemic, possibly due to the presence of the *P. berghei*. Mice that were infected and treated with artemether-lumefantrine and *G. kola* were healthy, but not anaemic. It was noted that as the number of days increased in the post-infection period, percentage suppression increased, except in the positive and negative controls. In the positive control group, there was a steady increase in the percentage parasitaemia, and no suppression was observed. However, the antimalarial component of the seed could have been responsible for the survival of the mice treated with the methanol extract of *G. kola* throughout the session of the experiment, when compared to the positive control group that died before the experiment was terminated. Interestingly, reports are indicating that *G. kola* has antimicrobial activity (Konziase,2015) and some authors have attributed the anti-plasmodium activity of *G. kola* to the presence of bioflavonoids. In earlier works of (Adaramoye et al.2019), it was discovered that the bioflavonoids have inhibitory activity *in vitro* against *P. falciparum* proliferation, and also, antimalarial potency in mice infected with *P. berghei*. Some authors have concluded that the antioxidant properties of kola viron from *G. kola* seeds inhibited oxidative damage (Atsukwei et al.2015). According to NENT,2018 a study on *G. kola* demonstrated that all the graded doses of the methanol extract of *G. kola* seed administered to mice showed no signs of toxicity, and no deaths was recorded. Therefore, the LD_{50} of the methanol extract of *G. kola* seed was found to be safe up to 5000 mg/kg *per* oral. On the weights of the animals (suppressive model), at weight 2, the weight increased when compared with the negative control group, which was found to be statistically significant, while in the curative model, the weights of animals that were administered the methanol extract of *G. kola* significantly increased relative to the negative control. As part of recommendation, isolation and characterisation of the bioactive compound(s) responsible for the antimalarial activity of *G. kola* are required. Sub-acute and chronic toxicity studies need to be conducted, especially in higher mammals, so as to conclusively establish the toxicity profile of the research plant.

CONCLUSIONS

The methanol extract of *G. kola* had a dose-dependent antimalarial activity against artemisinin-lumefantrine sensitive *P. berghei*. The effect on malaria parasites was demonstrated by its suppressive effect and confirmation by its curative effect.

Supplementary materials

The supplementary material / support for this article can be found online and downloaded at: https://www.isisn.org/article/

Author contributions

Conceptualisation and supervision, P.N.; Co-supervision, A.E., I.O., and K.N.; Collection of data/ bench work, B.N.; Review of data analysis, C.N.; Writing-original draft preparation, B.N. and P.N.; and Writing-up review and editing, C.N. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

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Data Availability Statement

All of the data is included in the article/supplementary material.

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Conflict of Interest

The authors declared no conflicts of interest.

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