ANTIPLASMODIAL POTENTIAL OF *Garcinia kola* (HECKEL) STEMBARK EXTRACT IN ALBINO MICE

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ABSTRACT

Garcinia kola stem bark forms part of recipes used traditionally for the treatment of malaria. In view of the prevalence of malaria in Nigeria, this study investigated the phytochemical, mineral and proximate components, as well as antiplasmodial activity and toxicological effect of *Garcinia kola* stem bark extract against *Plasmodium berghei* infected mice. The plant sample was screened for phytochemical, mineral and proximate components using standard laboratory techniques. Thirty five mice were divided into seven groups of five mice each. Malaria was induced in all the groups intraperitoneally with 0.2 mL of infected blood containing about 10^7 of *P. berghei* parasitized red blood cells, except group 6 (extract only) and group 7 (normal control). Group 1 received 100 mg/kg bodyweight of the extract orally. Group 2 received 200 mg/kg of the extract. Group 3 received 300 mg/kg of the extract. Group 4 received 5 mg/kg of chloroquine. Group 5 (induced but untreated control). The haematology, liver function enzymes and histopathology of the liver were carried out using standard protocols. The plant was rich in alkaloids, iron and fibre. The extract treated groups (1-3) showed significant decrease ($p \le 0.05$) in parasitemia level after seven days of treatment. There was no significant difference in AST, ALT, ALP, bilirubin and GGT activities in all the extract treated groups compared to the control. No pathological changes were evident in histopathology of all the groups treated with various concentration of the extract. The result obtained from this study confirmed the antiplasmodial activity of methanol extract of *G. kola* stem bark. The highest inhibition of *P. berghie* parasite was observed at dose 300 mg/kg comparable to chloroquine, with no hepatoxicity which confirmed the safety of *G. kola.* The phytochemicals and nutritional components could be responsible for the observed antiplasmodial activity of the plant.

Keywords: *Garcinia kola*, Phytochemical components, Nutritional values, *Plasmodium berghei*, Antiplasmodial effect.

INTRODUCTION

Malaria is undoubtedly one of the world's most deadly diseases (Winter *et al.*, 2006). It is a major public health problem in Nigeria where it accounts for more death cases than any other country in the world. In Nigeria, 97% of its population is at risk of malaria with an estimated 100 million malaria cases and over 300,000 deaths per year in Nigeria (Salihu and Sanni, 2013). Malaria is still Africa's leading health problem due to drug resistance to most anti-malaria drugs, insecticide resistance in mosquitoes, war and civil disturbances, environmental changes, climatic changes, travel and population increase (WHO, 2008).

Malaria is one of the major tropical parasitic diseases responsible for significant morbidity and mortality especially among children and pregnant women (Ekeanyanwu and Ogu, 2010). Malaria parasites belong to the genus Plasmodium (phylum Apicomplexa). In humans, malaria is caused by *P. falciparum, P. maglariae, P. ovale, P. vivax and P. knowlesi* (Mueller *et al.*, 2007). Severe malaria is usually caused by *P. falciparum* (often referred to as falciparum malaria).

Malaria is transmitted to humans via the bite of an infected female *Anopheles* mosquito. Only female mosquitoes feed on blood; male mosquitoes feed on plant nectar, and thus do not transmit the disease. The females of the Anopheles genus of mosquito prefer to feed at night. They usually start searching for a meal at dusk, and will continue throughout the night until taking a meal (Arrow *et al.*, 2004). The signs and symptoms of malaria typically begin 8-25 days following infection (Bartoloni and Zammarchi, 2012; Fairhurst and Wellems, 2010). The classic symptom of malaria is paroxysm, a cyclical occurrence of sudden coldness followed by rigor and then fever and sweating, occurring every two days (tertian fever) in *P. vivax* and *P. ovale* infections, and every three days (quartan fever) for *P. malariae. P. falciparum* infection can cause recurrent fever every 36–48 hours or a less pronounced and almost continuous fever (Ferri, 2009).

Drugs used in the treatment of malaria are, chloroquine, mefloquine, artesunate, artemether and bulaquine and their side effects include, vision impairment, deafness, loss of appetite, nausea, vomiting, diarrhoea, abdominal cramps, skin eruptions, pigmentation, itching, sensitivity to light, hair loss, anaemia, reversible decrease in white blood cells and platelets, headache, depression and low blood pressure. Other side effects are dizziness, muscle pain, fever, headache, chills, skin rash, fatigue, circulatory disturbances, chest pain, fast heart rate, rash, hives, and slow heart rate (Winstanley *et al.*, 2004).

Currently, multi-drug resistance has become one of the most important problems impeding malaria control efforts (Sendagire *et al.*, 2005; Htut and Engl, 2009). In the late 1940s, chloroquine was massively used and accepted worldwide, but resistance has spread to the vast majority of the malaria endemic regions like Africa, South East Asia and East Asia (Sanket and Sarita, 2009). Resistance to mefloquine has become an issue in Combodia, Myanmar, and some border areas of Thailand. Although quinine and tetracycline are used in combination for treating uncomplicated malaria in some areas like Brazil and South East Asia, sensitivity to quinine is still seriously diminishing (Fidock *et al.*, 2004). Hence, the problem of resistance of plasmodium to antimalarial drugs in the malaria endemic regions of the world has left this region with an unprecedented situation in which the few and affordable treatment options are rapidly losing therapeutic efficacy (Khozirah *et al.*, 2011). Spread of multidrug-resistant strains of plasmodium and the adverse side effects of the existing anti-malarial drugs have necessitated the search for novel, well tolerated and more efficient antimalarial drugs (Bickii *et al.*, 2000).

In Africa up to 80 % of the population still rely on herbal medicine to treat malaria and other diseases (Agbedahunsi, 2000), because of their affordability and accessibility. *Alstonia boonei* bark or leaves are administered as decoction (Majekodunmi *et al.*, 2008). Most anti-malarial plants are used in form of monotherapy, and only a few plants are taken together in combined therapies. An exam-

ple is the multi-herbal antimalaria remedy that includes *Cajanus cajan* leaf, *Euphorbia lateriflora* leaf, *Mangifera indica* leaf and bark, *Cassa alata* leaf, *Cymbopogon gigateus* leaf, *Nauclea latifolia* leaf, and *Uvaria chamae* bark (Nwabuisi, 2002). *Garcinia kola* has many therapeutic values in Nigeria. It is used for the treatment of bronchitis, throat infections, and as an aphrodisiac, antioxidant, hypoglycaemic, antipurgative and antiparasitic, anticandidal and antimalarial agent (Gbadamosi *et al.*, 2011; Ekene and Erhirhie, 2014).

In view of the prevalence of malaria in Nigeria and the traditional use of *Garcinia kola* stem bark as antimalaria herb, this study investigated the phytochemical, mineral and proximate components of the plant, as well as its *in vivo* antiplasmodial activity in mice. This was done with a view to providing scientific information on its efficacy as an antimalaria remedy.

MATERIALS AND METHOD
Collection, Identification and Preparation of Plant material
Garcinia kola was collected from the campus of the University of Ibadan. and identified at University of Ibadan Herbarium (UIH). The stem bark of the plant was washed, cut into small pieces and air dried at room temperature for two weeks and grounded to powder using electric blender. The powdered sample was put in an airtight bottle and stored at 4 ^{o}C for further use.

Phytochemical, Mineral and Proximate Analyses
The powdered plant sample was screened for the presence of alkaloids, saponins, tannins, phenols, glycosides, philobatannins and anthraquinones (Wolfe *et al.*, 2003; Harborne, 2005). The plant sample was digested prior to mineral analysis (Walsh, 1971). The sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu) and iron (Fe) contents were determined using atomic absorption spectrophotometer. Phosphorus (P) was analysed using vanadolybdate method and the absorbance was read at 400 nm (AOAC, 2005). The ash, crude fat, crude fibre, crude protein and moisture contents of the plant sample were analysed in the Laboratory of the Department of Animal Science, University of Ibadan, Nigeria (ASEAN, 2011).

Experimental Animals
Thirty five male Swiss albino mice (20-30 g) used for the experiments were obtained from the Department of Pharmacognosy Animal House, University of Ibadan, Nigeria. The animals were fed with standard feed and had free access to water. They were also maintained under standard conditions of humidity, temperature and 12h light/darkness cycle. The animals were acclimatized for two weeks before the commencement of the study. The experiment was conducted in accordance with the guidelines for the care and use of laboratory animals (NIH, 2002).

Maintenance and Estimation of plasmodium parasite in experimental animals
Plasmodium berghei (NK-65) strain was obtained from the Department of Biochemistry, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos. The *P. berghei* was subsequently maintained in the laboratory by serial blood passage from mice to mice every 5-7 days. Three animals at a time were used as infected donors and as parasite reservoir. The donor mice were monitored for signs of infection which include lethargy, anorexia, ruffled appearance, shivering and heat-

seeking behavior. Blood was taken from a donor mice previously infected with *P. berghie* (NK-65) and diluted with normal saline. The percentage parasitemia and red blood cell count were determined. The parasitemia was estimated by examination of a well stained blood film. This was then accomplished by counting the number of parasitized red blood cells (pRBC) seen in 10^4 red blood cells (equal to approximately 40 monolayer cell fields of a standard microscope using x100 oil immersion objective lens, each field containing approximately 250 RBCs). The number of pRBC was then divided by 100 to express it as a percentage (Moody, 2002).

Inoculation of Animal with parasite
Each mouse in the infected groups was inoculated intraperitoneally with 0.2 mL of infected blood containing about 1×10^7 of *P. berghei* parasitized red blood cells using a 1mL syringe. Blood smear was prepared, stained, and observed under the microscope to establish parasitemia on the fifth day after inoculation.

Preparation of methanol extract of sample and Chloroquine Phosphate
Extracts were prepared by maceration of the powdered plant material with methanol; The powdered sample (750 g) was macerated with 2.5 L of 50 % methanol for 24 hours. After stirring for 3 hours, the methanol was separated by filtration and then maceration was repeated three times on the residue. The three macerates were pooled and concentrated by a rotary evaporator at 30°C to obtain a 60 g of methanol extract. The extract was kept in a tightly closed bottle in a refrigerator for further use. The extracts were prepared into 100, 200 and 300 mg/kg concentrations that were used for the antiplasmodial activity in mice. Two

tablets each containing 250 mg chloroquine phosphate equivalent to 150 mg chloroquine base were dissolved in 50 mL distilled water and the resulting solution was shaken until a homogeneous solution was obtained. The animals were given 40 µL of the solution which is equivalent to 5 mg/kg body weight. The extract and standard drug were administered into the experimental mice orally during the experiment.

Experimental Set-up
The animals were divided into seven groups and each group consisted of 5 mice, the groups were treated as follows:

Group 1 (G1): They were infected with *P. berghei* and treated with 100mg/kg of *G. kola* stem bark.

Group 2 (G2): They were the *P. berghei* infected group treated with 200mg/kg of *G. kola* stem bark.

Group 3 (G3): They were the *P. berghei* infected group treated with 300mg/kg of *G. kola* stem bark.

Group 4 (G4): They were the *P. berghei* infected group treated with 5mg/kg of chloroquine.

Group 5 (G5): They were the *P. berghei* infected but not treated they were given access to normal feed and water.

Group 6 (G6): They were not infected with *P. berghei* but were given 200mg/kg of *G. Kola*, which served as the extract control group.

Group 7 (G7): The control group that were neither infected nor treated throughout the experiment but were given free access to feed and water.

Estimation of parasitemia

A drop of tail blood was collected from the mice into microscopic slides. A glass spreader was then used to spread the blood into a thin film. The spreader was drawn back to touch the drop of blood and the blood was allowed to extend along the edge of the spreader. Holding the spreader at about 30^0, the drop of blood was spread to make a film of about 40-50 mm in length (two-third of the slide) (Cheesbrough, 1998). The film was allowed to dry, and then fixed with absolute methanol. The fixed film was then air dried and stained with 10% Giemsa stain at pH 7.2 for about 15 minutes after which excess stain was washed off under a running tap water and allowed to dry. A drop of immersion oil was then placed on the slide and viewed under the microscope using the X100 oil immersion objective lens.The parasitemia was monitored in all the groups starting from day 5 to day 11 and estimated by examining a well of stained thin blood films made from the tail vein of the mice. This was accomplished by counting the number of pRBC seen in 10^4 RBC using the X100 oil immersion microscope.

The % parasitemia was then calculated as follows

$$\%parasitemia=\frac{total\ number\ of\ pRBC\ X100}{total\ number\ of\ RBC}$$

Estimation of % suppression parasitemiain mice

The percentage suppression of parasitemia was calculated for each dose level by comparing the parasitemia in infected controls with those of treated mice as follows:

$$\%\ Suppression = \frac{APN-APP}{APN}$$

Where,
APN= Average % parasitaemia in negative control

APP= Aaverage % parasitaemia in positive groups
APN= Average % parasitaemia in negative control

Blood collection, Dissection and Collection of Organ

At the end of the experiment, blood samples were collected from each surviving mice of the study by eye puncture method. The mice were gently held with the ventral side facing up by applying pressure on the dorsal skin fold close to the head region. The cornea of the eye was then punctured by means of a sterile capillary tube while the blood was collected immediately into heparinised tubes. The sample bottles were gently rocked to allow a proper mixing of the blood with the anticoagulants in the sample bottles which were properly labelled. The animals were then sacrificed according to guides by Rowett (1977). The liver was then collected and stored in 10 % formalin for further histopathological studies.

Haemathological Analysis of blood samples

The Packed Cell Volume (PCV), Heamogloubin (Hb), Red blood cell (RBC), White blood cell (WBC), lymphocytes, neutrophil, monocyte and eosinophil were determined using standard methods (Ashour *et al.*, 2007).

Biochemical Analysis

The biochemical parameters were determined using standard biochemical kit. Parameter assay includes Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP) and Gamma Glutamytanspeptidase (GGT) (Kutty and Jacob, 2004).

Histopathological studies

The liver was collected from each sacrificed animal and stored in 10 % formalin in properly labelled sample bottles. The organs were thereafter dehydrated by passing them through increasing concentration of ethanol 70 %, 80 %, 90 % and 100 % one hour each after the other to remove water from the tissues. Thereafter, the organ was impregnated in three changes of molten wax to remove xylene from the tissue and to replace it with paraffin wax. The organs were later embedded in molten wax and allowed to solidify. The blocks were mounted on the microtome and sectioned at 5 microns. The histopathological analysis of the liver of animals was done using the method of (Morton *et al.*, 1997).

Data Analysis

The results were expressed as mean ± SE. The data was analyzed using ANOVA and Duncan multiple range test, differences between mean were considered significant at ($P< 0.05$), using Statistical Package for Social Sciences (SPSS) version 17.0 computer software.

RESULTS

Results of the phytochemical screening of *G. kola* stem bark showed that it contained alkaloids, saponins, tannins, phenol, glycosides, philobatannins and anthraquinones (Table 1). The iron content (38.45 %) of the plant was the highest of its mineral components, followed by zinc (31.50 %), cupper (6.60 %) while calcium (0.04 %) and sodium (0.04 %) were the least (Table 2). The fibre (41.81 %) content of the plant sample was the highest, followed by moisture (9.96 %), protein (8.83 %) with fat (2.65 %) being the least (Table 3). In Figure 1, the mean body weight of all *Plasmodium berghei* infected mice decreased before and after treatment (Groups 1 - 5). A decrease in body weight was also observed in the groups treated with plant extract only (Group 6), whereas there was significant increase in the mean body weight of the control group (Group 7). The antiplasmodial effect of *G. kola* extract and chloroquine in mice is presented in Table 4. The extract significantly reduced PVC, Hb and RBC values in mice (Table 5). The treatment ameliorated the adverse effect on biochemical parameters of treated groups when compared to the untreated group (Table 6). Fig 2 shows the effect of the extract and standard drug on the liver of the mice.

Table 1: Pytochemical components of *Garcinia kola* stembark

`Phytochemical	Qualitative
Alkaloids	+++
Saponins	+++
Tannins	++
Phenols	+++
Glycosides	+++
Anthraquinones	++

Legend: += Present

**Table 2: Mineral components of *Garcinia kola* stembark**

Mineral	Composition (%)
Sodium	0.04 ± 0.12^e
Potassium	0.11 ± 0.12^{de}
Calcium	0.04 ± 0.12^{de}
Phosphorus	0.22 ± 0.12^{de}
Magnesium	0.30 ± 0.05^d
Zinc	3.15 ± 1.00^b
Cupper	0.66 ± 1.00^c
Iron	3.84 ± 1.00^a

Values are mean $\pm$ SE, n = 2, Values having different superscript were considered significantly different ($P < 0.05$) in quantity.

Table 3: Proximate contents of *Garcinia kola* stembark

Parameters	Composition (%)
Protein	8.83 ± 1.00^b
Fat	2.65 ± 1.00^e
Ash	3.15 ± 1.00^d
Fibre	41.81 ± 1.00^a
Moisture	9.96 ± 1.00^b

Legend: Values are mean $\pm$ SE, n = 2, Values having different superscript were considered significantly ($P < 0.05$) different in quantity.
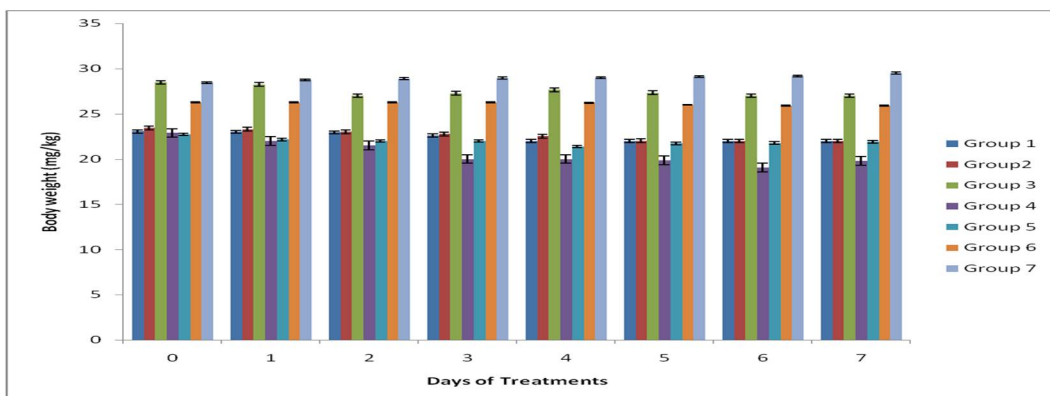
Figure 1: Effect of treatment on mean body weight of the *P. berghei* infected and control mice.

Group G1 (100 mg/kg of *G. kola* extract), Group G2 (200 mg/kg of *G. kola* extract), Group G3 (300 mg/kg of *G. kola* extract), Group G4 (5 mg/kg of chloroquine), Group G5 (infected but not treated), Group G6 (200 mg/kg of *G. kola* extract only), Group G7 (normal control). Day 0: Initial body weight before infection; Day1-7: Days of treatment after infection.

Table 4: Suppressive effect of *G. kola* extract and chloroquine against *P. berghei* infection in mice

Group	Parasitemia Count					Suppression (%)	Mortality (%)
	Induction	Day 1	Day 3	Day 5	Day 7		
G1	18.80±5.16*	17.92±4.47	6.56±2.16*	4.24±2.15	2.40±0.58*	33.60±2.95	20
G2	19.92±1.37	19.28±6.28	5.44±1.97	3.00±0.59*	1.62±0.36*	95.18	20
G3	18.68±1.75	8.02±3.38	3.86± 0.57	2.66±0.37*	1.60±0.38*	95.24	20
G4	17.92±4.79	7.44±1.52*	5.12±1.26	3.00±0.27*	0.96±0.56*	97.14	20
G5	15.16±1.37	17.92±4.46	22.70±0.75	31.20±3.42	33.60±2.95	0.0	80
G6	-	-	-	-	-	0	-
G7	-	-	-	-	-	0	-

Legend: Values are mean ± SE, n =5; *was considered significantly different compared with control group at P < 0.05.
Chloroquine (CQ). Group G1 (100 mg/kg of *G. kola* extract), Group G2 (200 mg/kg of *G. kola* extract), Group G3 (300 mg/kg of *G. kola* extract), Group G4 (5 mg/kg of chloroquine), Group G5 (infected but not treated), Group G6 (200 mg/kg of *G. kola* extract only), Group G7 (normal control).

Table 5: Effect of *Garcinia kola* extract and Chloroquine on the hematological parameters of *P. berghei* infected mice

Group	PVC (%)	Hb (g/dl)	RBC (×10⁶/µl)	WBC (×10³/µl)	Platelets (×10³/µl)	Lymp (%)	Neutro (%)	Mono (%)	Eosino (%)
G1	17.67± 0.54^b	5.87±0 .32^b	2.96±0.3 0^b	7.33±0.1 2^{ab}	139.00±0. 12^a	74.00± 0.31^{ab}	22.00± 0.08^{ab}	2.67±0 .05^{ab}	1.00±0. 12^{bc}
G2	23.00± 0.54^b	7.00±0 .32^b	3.42±0.3 0^b	8.00±0.0 5^a	104.00±0. 12^{ab}	81.00± 0.06^a	16.00± 0.08^b	2.00±0 .05^{ab}	1.00±0. 12^{bc}
G3	24.33± 0.54^b	6.45±0 .32^b	3.35±0.3 0^b	8.00±0.0 5^a	101.00±0. 07^{ab}	74.00± 0.31^{ab}	25.00± 0.08^{ab}	1.00±0 .05^b	0.00±0. 12^c
G4	37.33± 0.83^a	12.20± 0.45^a	6.05±0.4 5^a	6.67±0.1 2^b	110.33±0. 07^{ab}	72.67± 0.31$_{ab}$	24.67± 0.08^{ab}	1.33±0 .05^{ab}	1.33±0. 13^b
G5	19.33± 0.54^b	6.33±0 .32^b	3.09±0.3 0^b	7.67±0.1 2^{ab}	134.33±0. 12^a	68.33± 0.31^b	26.67± 0.08^{ab}	2.33±0 .05^{ab}	2.67±0. 10^a
G6	36.67± 0.83^a	11.40± 0.45^a	5.86±0.4 5^a	6.00±0.1 2^{ab}	123.33±0. 12^a	69.33± 0.31^{ab}	26.00± 0.08^{ab}	3.00±0 .05^a	1.67±0. 13^{ab}
G7	37.00± 0.83^a	12.23± 0.45^a	6.18±0.4 5^a	6.67±0.1 2^{ab}	68.00±0.0 7^b	68.00± 0.31^b	28.67± 0.26^a	2.67±0 .05^{ab}	0.67±0. 12^{bc}

Legend: Values are mean ± SE, n = 5; Values having the different superscript were considered significantly different at P < 0.05.
Packed Cell Volume (PCV), HeamLgloubin (Hb), Red blood cell (RBC),White blood cell (WBC), Lymphocytes (Lymp), Neutrophil (Neutro), Monocyte (Mono), Eosinophil (Eosino), Chloroquine (CQ). Group G1 (100 mg/kg of *G. kola* extract), Group G2 (200 mg/kg of *G. kola* extract), Group G3 (300 mg/kg of *G. kola* extract), Group G4 (5 mg/kg of chloroquine), Group G5 (infected but not treated), Group G6 (200 mg/kg of *G. kola* extract only), Group G7 (normal control).

Table 6: Effect of extract of *G. kola* and chloroquine on the liver enzymes of *P. berghei* infected mice

Group	AST (UI/L)	ALT (UI/L)	ALP (UI/L)	BILIRUBIN (µmol/l)	GGT(UI/L)
G1	204.67±0.13[ab]	108.67±0.32[b]	135.67±0.24[a]	0.37±0.07[ab]	3.83±0.19[a]
G2	170.00±0.13[ab]	130.00±0.05[ab]	153.00±0.24[a]	0.40±0.07[ab]	3.80±0.19[a]
G3	144.50±0.13[b]	101.50±0.32[b]	120.50±0.24[a]	0.15±0.05[b]	3.00±0.19[a]
G4	231.67±0.13[a]	125.67±0.23[b]	134.67±0.24[a]	0.27±0.05[ab]	3.80±0.19[a]
G5	189.00±0.13[ab]	186.00±0.05[a]	154.33±0.24[a]	0.20±0.05[ab]	3.10±0.19[a]
G6	194.33±0.13[ab]	130.67±0.05[ab]	130.67±0.24[a]	0.30±0.05[ab]	4.07±0.19[a]
G7	206.00±0.13[ab]	116.33±0.32[b]	140.67±0.24[a]	0.40±0.07[a]	4.03±0.19[a]

Legend: Values are mean ± SE, n = 5. Values having the different superscript from the control were considered significantly different at P-value < 0.05

Chloroquine (CQ), Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP) Gamma Glutamytanspeptidase (GGT). Group G1 (100 mg/kg of *G. kola* extract), Group G2 (200 mg/kg of *G. kola* extract), Group G3 (300 mg/kg of *G. kola* extract), Group G4 (5 mg/kg of chloroquine), Group G5 (infected but not treated), Group G6 (200 mg/kg of *G. kola* extract only), Group G7 (normal control).
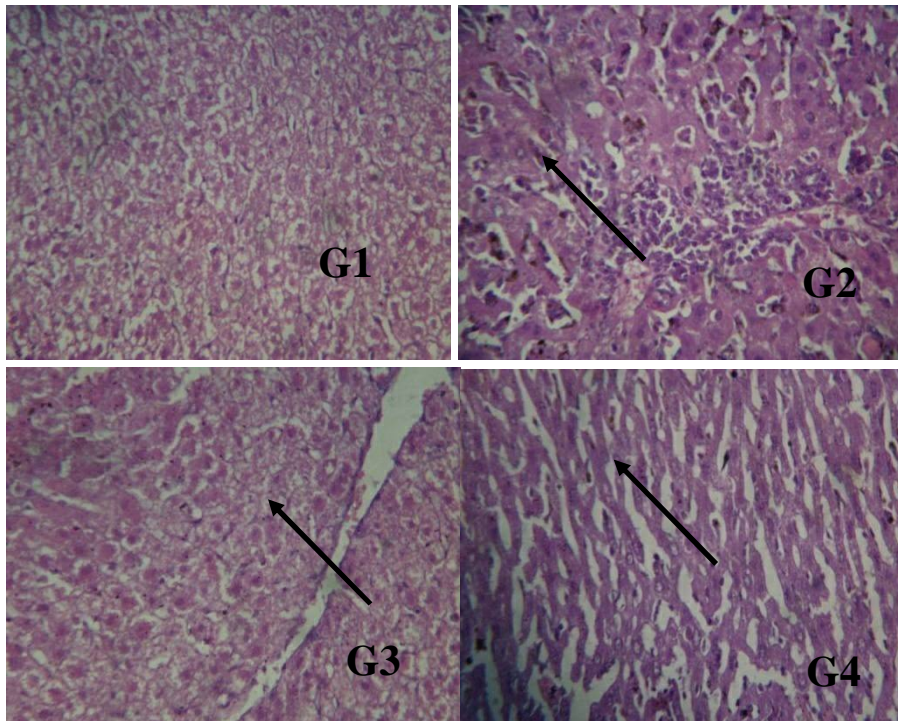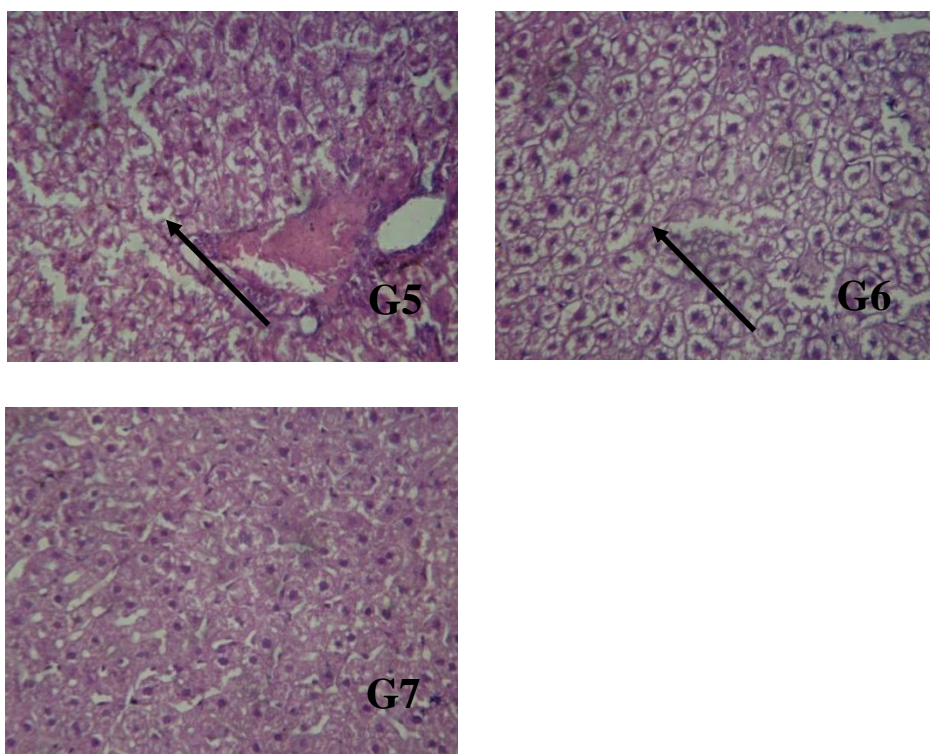
Figure 2: Histopathology of liver of control and treated groups of mice (Magnification x 400).

G1(100 mg/kg of *G. kola*): There was widespread uniform severe vacuolar change of the hepatocytes.

G2 (200 mg/kg of *G. kola*): There was moderate thinning of hepatic cords and multiple foci of Kupffer cells with intracytoplasmic brownish yellow pigments. G3 (300 mg/kg of *G. kola*): There was diffuse moderate vacuolar change of the hepatocytes. G4 (5 mg/kg chloroquine): There was moderate widespread thinning of hepatic cords and mild mononuclear perivascular cuffs. G5 (untreated mice): There was widespread vacuolar change of the hepatocytes with increasing severity towards the peri-portal areas; also mononuclear cellular aggregates in the peri-portal areas. G6 (extract only 200 mg/kg): There was moderate vacuolar change of the hepatocytes. G7 (control): The hepatocytes had a finely vacuolated (foamy) appearance (no visible lesion).

DISCUSSION

G. kola was rich in phytochemicals such as alkaloids, saponins, tannins, phenols and anthraquinones. The therapeutic activities of medicinal plants are attributed to their bioactive components (Chindo *et al.*, 2003). The literature is replete with information on antiplasmodial activity of plant derived alkaloids such as oxyacanthine, alstonerine, and cryptolepine (Saxena *et al.*, 2003). In the present study, the phytochemicals might be responsible for the antiplasmodial activity of *G. kola* stem bark. Furthermore, the mineral and nutritional components of the plant

could serve as supplements to the phytochemicals in their antiplasmodial activity.

A decrease in body weight was observed in all infected groups. The decrease in body weight might be due to decrease in food intake owing to loss of appetite caused by malaria. The loss in body weight could be as a result of disturbed metabolic function and hypoglycaemia that are reportedly associated with malaria infection (WHO, 2011). The group that was treated with plant extract (200mg/kg) only also had a decrease in weight compared to the control group. This is an indication that *G. kola* stem bark could be valuable in the management of obesity. The finding of the present study agrees with an earlier report on decrease in body mass in rats fed with *G. kola* (Uko *et al.*, 2001). The weight loss effect of the plant could be attributed to its high fibre content. Dietary fibres are employed in the treatment of diseases such as obesity, diabetes, cancer and gastrointestinal disorders (Saldanha, 1995).

The methanol extract of *G. kola* showed anti-plasmodial activity against *P. berghei* infected mice based on percentage of parasite inhibition (94.55 - 95.24 %), the % parasite inhibition was in the order: Group 4 (97.14 %) > Group 3 (95.24 %) > Group 2 (95.18 %) > Group 1 (94.55 %). The chloroquine (5mg/kg) treated group (Group 4) was the most effective and 300mg/kg of extract (Group 3) was the most active treatment. The result of the antiplasmodial activity of *G. kola* stem bark recorded in this study evidenced by the significant suppression of parasitemia in the extract treated groups is in line with the reports of previous authors (Valsaraj *et al.*, 1995). The observation is a further validation of the traditional use of the plant as herbal remedy for the treatment of malaria in Nigeria.

In the present study, a marked reduction in the RBC, PVC and Hb was observed in untreated plasmodium infected rats, however, rats tested with methanol extract of *G. kola* stem bark showed dose dependent increase of these parameters. Anaemia is usually assessed by evaluating PCV, Hb and RBC counts and previous authors have reported a progressive fall in RBC, PCV and Hb values in plasmodium infections (Ajagbonna *et al.*, 2002). The increase in haematological parameters is an attestation to the haematopoietic potentials *G. kola* stem bark. The slight increase in platelet count recorded in all *P. berghei* infected mice might be as a result of the short period of infection; it is possible that prolonged or repeated infections may lead to thrombocytopenia. White blood cells (WBCs), as well as other cells, are involved in the body's immune system and help to fight diseases. They are increased in normal situations such as exertion and pregnancy, and abnormally in situations such as loss of blood, cancer, and most infections. An increase in WBCs has been demonstrated to be linked to severe malaria (Modiano *et al.*, 2001).

There was no significant difference in AST, ALT, ALP, bilirubin and GGT activities in all treated groups compared to the control group. Liver cell damage is characterized by a rise in plasma enzymes of AST, ALT, ALP and GGT levels (Adaramoye *et al.*, 2008). The values of the plasma enzymes indicated no liver damage in all the groups treated with various doses of *G. kola* extract and standard drug. This finding confirms that *G. kola* is not hepatotoxic. The rate of mortality observed in the infected but untreated group (Group 5) was 80 %, this was observed from the sixth day after infection with *P. berghei.* Massive lost in the total number of normal

RBC in this model showed a characteristic of severe sign of anaemia which is one of the major clinical manifestations of severe malaria in human. The pathogenesis of severe anaemia during malaria infection is complex and involves multiple processes relating to both the destruction and decreased production of erythrocytes (Menendez et al., 2000).

The histological studies revealed that there was severe damage in the liver of the infected but untreated group (Group 5). The damage could be as a result of parasite life cycle during erythrocytic stages in the blood stream. At the early stage of the exoerythrocytic phase, a parasite infects the hepatocyte and multiplies asexually releasing merozoites which rupture their host cells. The liberated merozoites leave the liver and enter the blood stream where they infect the red blood cells (Siddiqi et al., 2002). The infected groups treated with plant extract (Groups1 to 3) showed a moderate widespread thinning of the hepatic cords; widespread vacuolar change of the hepatocyte and moderate vacuolar change of periportal hepatocytes. This may be due to the brief delay before parasite clearance, that is, the period before treatment. The uninfected group (G6) treated with plant extract only had a moderate thinning of the hepatic cords; and moderate vacuolar change of the hepatocytes. *Garcinia kola* has protective effect against a variety of experimental hepatotoxins (Akintonwa and Essien, 1990). This confirms that consumption of *G. kola* stem bark is not toxic to the liver which is the first organ susceptible to any injurious substance in case of toxicity. The control group (G7) had normal hepatocytes and a few foci of moderate aggregates of mononuclear cells.

CONCLUSION

Garcinia kola stem bark showed considerable antiplasmodial activity against *P. berghie* infected mice and was best at 300mg/kg with no toxic effect. Further studies should include purification of active components with the view to using the plant as an antimalarial agent.

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