

Original Research Article

Phytochemical Screening and Antiplasmodial Activity of Ethanolic Bark Extract of *Khaya grandifoliola* in Swiss Albino Mice Infected with *Plasmodium berghei* NK65.

ABSTRACT

Irrespective of the decreased incidence and prevalence, malaria remain a major public health problem. Evolution and spread of resistance to the available antimalarial drugs endanger all the recent gains in malaria control. This issue makes the development of novel drugs a necessity.

The key source in search of such drugs is medicinal plants (*Khaya grandifoliola*). *Khaya grandifoliola* is used in Ado Ekiti for management of malaria, but, no scientific investigations have been carried out to substantiate the usage. Thus, this study assessed the bioactive components and antiplasmodial activity of ethanolic bark extract of *K. grandifoliola*. Standard methods were employed to determine the bioactive components of the bark extract. 24 mice were randomly selected into six groups of four mice each (group 1, 2, 3, 4, 5 and 6) for antiplasmodial activity. The *P. berghei* infected groups were treated with *K. grandifoliola* extract with 0.2mL of 200mg/kg, 400mg/kg and 600mg/kg body weight respectively. Group 1 (negative control) was infected with *P. berghei* and administered with 0.2 mL of normal saline, Group 2 (positive control) were treated with 0.2mL of 5mg/kg body weight of chloroquine while group 3 (normal control) was not infected and administered with 0.2mL of normal saline for four consecutive days. Phytochemical Screening of ethanolic bark extract of *K. grandifoliola* contained alkaloids, saponins, tannins and anthraquinone. The extract treated groups 4, 5 and 6 revealed decrease in percentage parasitaemia compared with group 1 (infected and not treated). The parasitaemia reduction was high in group 6 (600mg/kg). The significant decrease ($P < 0.05$) in percentage parasitaemia was dose and time dependent. This result indicates that *K. grandifoliola* has a promising antiplasmodial activity and it could be considered as a potential source to develop new antimalarial agents.

Keywords: *Khaya grandifoliola*; *Plasmodium berghei*; antiplasmodium; phytochemical screening; albino mice.

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1.0

INTRODUCTION

Malaria is a mosquito-borne infectious disease affecting humans and other animals, caused by parasitic protozoa belonging to the *plasmodium* type. These parasites are spread to people through the bite of infected female *Anopheles* mosquitoes WHO [1].

According to WHO [1], the five species of *Plasmodium* that cause malaria in human are: *P. falciparum*, *P. Vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. *P. falciparum* is the most common type which cause malaria disease in humans. *P. knowlesi*, this species of the *Plasmodium* rarely causes disease in human.

In the work of Caraballo [2], malaria is most commonly transmitted by infected saliva of female *Anopheles* mosquito into uninfected person's blood. The symptoms are usually accompanied with fever, tiredness, shivering, vomiting, headache, yellow skin, seizures, coma, or death. In several cases, the symptoms usually begin 10- 15 days after being bitten with *P.*

falciparum. The risk of this disease can be reduced by preventing mosquito bites through the use of mosquito nets and insects' repellents or with mosquito control measure such as spraying insecticides and standing water Carabello [2]. Several medications are available to prevent malaria in travelers to areas where the disease is common WHO [1]. Occasional doses of the combination medication Sulfadoxine pyrimethamine are recommended in infants and after the first trimester of pregnancy in areas with high rates of malaria. The recommended treatment for malaria is a combination of antimalaria medications WHO, 2014[1]. Several years ago, antimalaria drug such as chloroquine, amodiaquine, mefloquine were effectively used to treat malaria, however the evolution and spread of resistance to these drugs is of major public health concern, hence, studies on medicinal plants could come out with useful information for the production of important active and non-toxic compounds as antimalarials. This research work is aimed at determining the phytochemical component of the ethanolic bark extracts of *K. grandifoliola* and its antiplasmodial effect in swiss albino mice infected with *Plasmodium berghei*.NK 65.

2.0

MATERIALS AND METHODS

2.1

Plant bark Collection

The fresh stem bark of *K. grandifoliola* also known as oganwo in Yoruba and Africa Mahogany in English Language were collected from Iboropa Akoko Ondo State South Western Nigeria, it was identified and authenticated by an expert in the Department of Biology, the Federal University of Technology, Akure, Nigeria. The voucher specimen number of the plant Bio/FUTA/ 700 was left in the herbarium of the Federal University of Technology, Akure, Ondo State, Nigeria.

2.2 Extraction and Phytochemical Screening of the Bark

Bankole [3] was adopted for the extraction. The bark plant was washed chopped into pieces with mortar and pestle and sun dried for two to three weeks, then stored in an air tight bottle before the analysis. Five hundred grams (500 g) of the grinded powder was soaked into 2.5 litres of 75% ethanol, stirred and left for 72 hours with continues shaking. The mixture was filtered using a whatman filter paper (pore size 0.7 μ m), the reddish brown was concentrated into dryness in a ceramic container using water bath at 40°C to obtain extract free of solvent and was refrigerated at -4°C for further studies.

2.3 Determination of Phytochemicals

The method of Bankole [3], was used to carry out phytochemical screening on powdered samples.

2.4 Preparation of Bark Extracts Dosages

The dosages of the extract administered to the mice were prepared by dissolving 1, 2 and 3 g of the extract in 5mL of distilled water each in sterile universal bottle to obtain 200, 400 and 600 mg/kg respectively Ogundolie *et al* (4).

2.5 Assemblage of Experimental Mice

Mice weighing between 19-23 g were obtained from animal house, Institute of Advance Medical Research and Training (IMRAT), University College Hospital (UCH) Ibadan. Mice were housed in plastic cages with saw dust beddings. The animals were fed with pellets, clean water and acclimatized for 7 days at room temperature 29°C -30°C before the commencement of the experiment. *Plasmodium berghei* NK 65 in a donor mouse was obtained from IMRAT.

2.6 Grouping of Animals

A sum of 16 mice were used for acute toxicity test and was grouped into four each, while a total of 24 mice were divided into six groups of four mice per group for antiplasmodium activity. Group 1 (not treated), group 2 (chloroquine treated group), group 3 (normal control), groups 4, 5 and 6 (extract treated groups) Ogundolie *et al.* (4).

2.7 Acute Toxicity

Acute toxicity test of the stem bark extract was carried out using Irungu [5]. Each mouse was respectively administered orally with 0.2ml (200, 400, 800, 1000 mg/kg body weight) of *Khaya grandifoliola* ethanolic bark extract. The mice were observed for seven days for mortality, body weakness, hyper activity; reduce activity, licking paw, salivation, inactiveness and death.

2.8 Preparation of Inoculum

Donor mouse of 20% parasitaemia was anaesthetized with chloroform 0.2 mls of the parasite was withdrawn from the infected mouse and serially diluted with sterile 4.8 mls of normal saline to obtain 1×10^7 *Plasmodium berghei* infected erythrocyte, Ogundolie *et al.* (4)

2.9 In vivo Antiplasmodial Activity of the Extract

Antiplasmodial activity of the extract was carried out using the petter's 4 days suppressive test as adopted by Dada and Oloruntola [6]. Twenty-four mice were randomly distributed into six groups of four mice per group. They were infected intravenously with 0.2 ml of 1×10^7 standard inoculum of chloroquine sensitive *P. berghei*. Four hours after infection, 0.2 mL of 200, 400 and 600 mg/kg body weight of bark extracts were administered orally to group 4, 5 and 6 respectively as treatment dose once daily for four consecutive days. Group 2 which is the positive control were treated with 0.2 mL of 5 mg/kg body weight of chloroquine, Group 1 were given 0.2 ml of normal saline which is the negative control and Group 3 which is normal control received 0.2 ml of normal saline but were not infected with *P. berghei*.

2.10 Determination of Parasitaemia

The method described by Bankole [3] was used. On day 5 of the experiment, 2 drops of blood sample were respectively collected onto grease free microscopic slides from the mice caudal vein. Thick and thin blood smear were made and allowed to air-dry. The air-dried films were fixed with 75% ethanol for 2-3 minutes, after which they were stained with 10% Giemsa stain for 15 minutes. The blood smear samples were rinsed with buffer distilled water PH 7.2.

The parasitaemia was determined by counting the part of the film where the white cells and parasites were evenly distributed. Using oil immersion objectives (X100), white blood cells were counted systematically; hand tally counter was used to count at the same time to know the parasite present in the fields.

% Parasitemia = (Number of parasitized RBC X100 / Total Number of RBC examined).

2.11 Statistical Analysis

All data were expressed as mean $\pm$ S.E. One-way analysis of variance was used to analyze data. $P < 0.05$ was considered significant difference between means (Duncan's multiple range test).

3.0 RESULTS

3.1 Phytochemical Screening Ethanolic Bark Extract of *K. grandifoliola*.

Phytochemical screening of ethanolic bark extract of *K. grandifoliola* revealed the presence of alkaloids, saponins, tannins, anthraquinone, flavonoid and carbohydrate.

3.2 Acute Toxicity of Ethanolic Bark Extract of *K. grandifoliola*

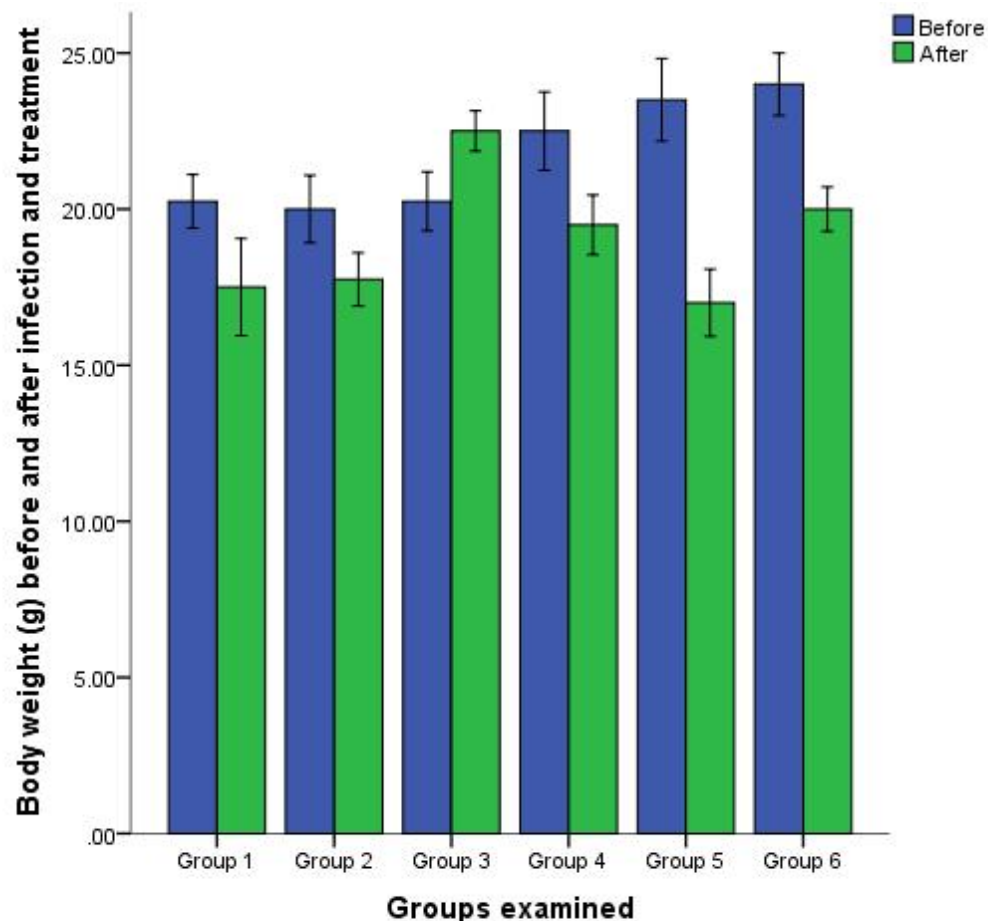
No sign of toxicity such as body weakness, hyperactivity, salivation, jumping, reduced activity, licking paw, convulsion, in the mice and no mortality was recorded for all the doses tested. The result indicated that the LD₅₀ is greater than 1000 mg/kg body weight.

3.3 The Effect of Bark Extract of *K. grandifoliola* on Body Weight of Mice Before and After Infection with *Plasmodium berghie*

The body weight of the group 1 and 2 (infected untreated and infected treated with 5 mg/kg chloroquine) showed no significant difference $P>0.05$ after 4 days of treatment (Figure 1). However, the mice treated with 400 mg/kg (group 4) had the highest decreased in body weight compared with those treated with 200 mg/kg and 600 mg/kg (group 4 and 6) after 4 days of treatment. Mice in group 3 (normal saline) had increased body weight.

Table 1: Result of Photochemical Screening of Ethanolic bark extract of *K. grandifoliola*

Plant name <i>K. grandifoliola</i>	Result
Alkaloid	+
Flavonoid	-
Saponins	+
Phenols	+
Carbohydrate	+
Anthraquinone	+



4.9.6 Fig 1 Effect of bark extract on parasitemia count of Swiss Albino Mice infected with *Plasmodium berghei*

The figure 2 showed parasitaemia count (%) of mice infected with *P. berghei* and treated with bark extract of *K. grandifoliola* for day 1; Figure 3 showed day 2 of the parasitaemia level of mice infected with *P. berghei*, Figure 4 showed parasitaemia level of Swiss Albino mice infected with *P. berghei* treated with bark extract of *K. grandifoliola* +5mg/kg chloroquine body weight +normal saline for day 3 and figure 5 showed the parasitaemia level of Swiss Albino mice infected with *P. berghei* + normal saline+ *K. grandifoliola*+5mg/kg chloroquine for day 4 and figure 6 showed day 5.

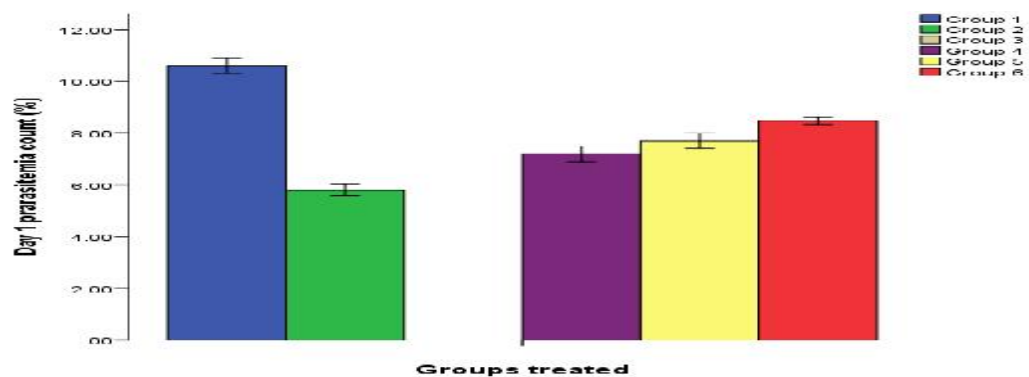


Figure 2: Parasitemia Count (%) for Day 1

Group 1: *P. berghei* + 0.2 ml normal saline, group 2: *P. berghei* + 5 mg/kg body weight Chloroquine, group 3: 0.2 ml normal saline, group 4: *P. berghei* + 200 mg/kg body weight bark extract, group 5: *P. berghei* + 400 mg/kg body weight bark extract and; group 6: *P. berghei* + 600 mg/kg body weight bark extract

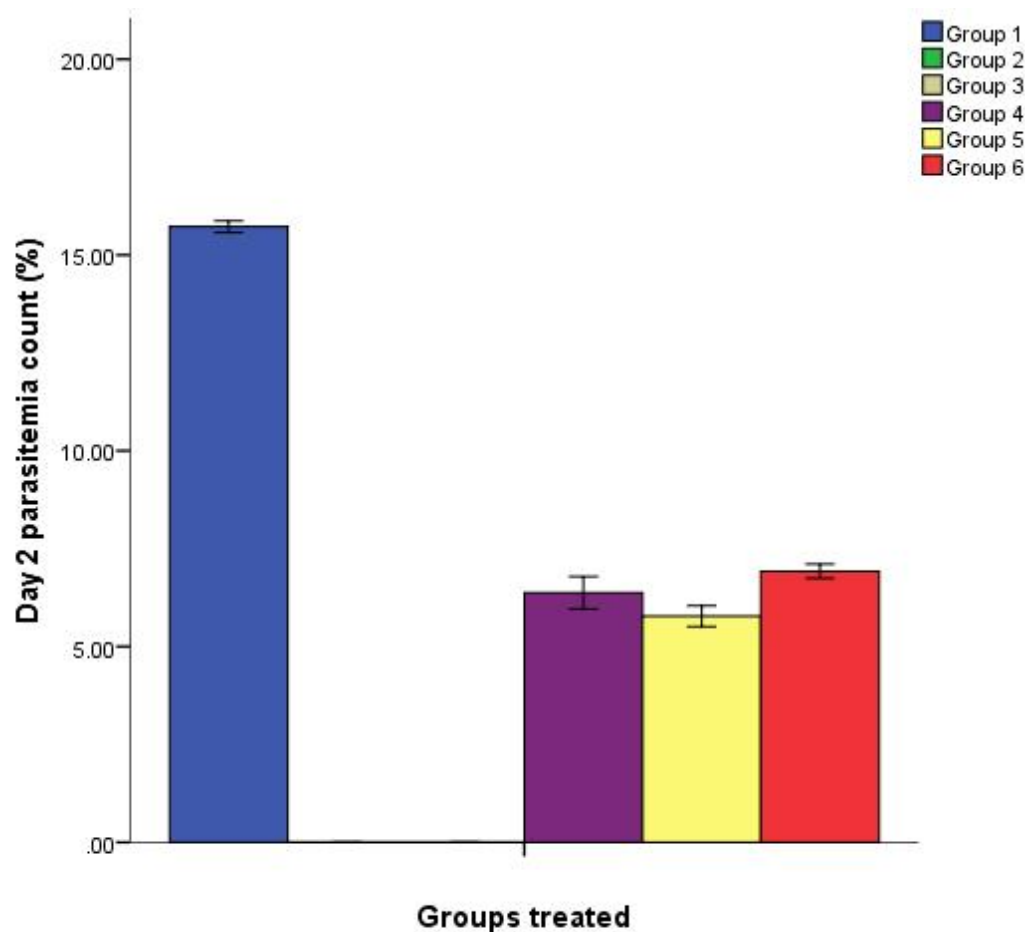


Figure 3: Parasitemia Count (%) for Day 2

Group 1: *P. berghei* + 0.2 ml normal saline, group 2: *P. berghei* + 5 mg/kg body weight Chloroquine, group 3: 0.2 ml normal saline, group 4: *P. berghei* + 200 mg/kg body weight bark extract, group 5: *P. berghei* + 400 mg/kg body weight bark extract and; group 6: *P. berghei* + 600 mg/kg body weight bark extract

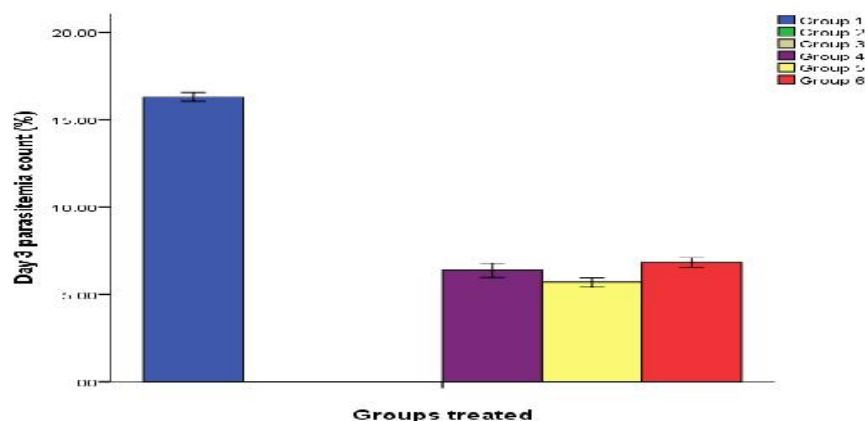


Figure 4: Parasitemia Count (%) for Day 3

Group 1: *P. berghei* + 0.2 ml normal saline, group 2: *P. berghei* + 5 mg/kg body weight Chloroquine, group 3: 0.2 ml normal saline, group 4: *P. berghei* + 200 mg/kg body weight bark extract, group 5: *P. berghei* + 400 mg/kg body weight bark extract and; group 6: *P. berghei* + 600 mg/kg body weight bark extract

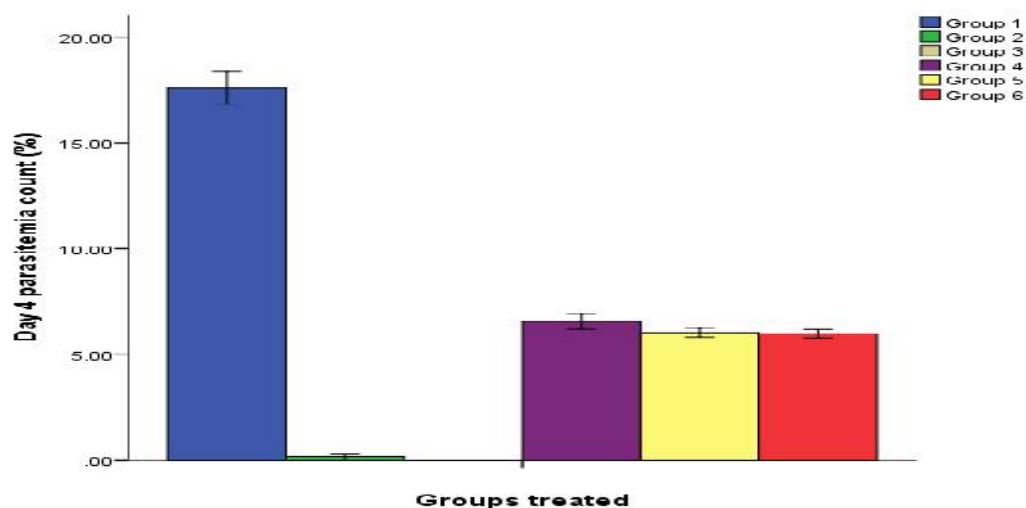


Figure 5: Parasitemia Count (%) for Day 4

Group 1: *P. berghei* + 0.2 ml normal saline, group 2: *P. berghei* + 5 mg/kg body weight Chloroquine, group 3: 0.2 ml normal saline, group 4: *P. berghei* + 200 mg/kg body weight bark extract, group 5: *P. berghei* + 400 mg/kg body weight bark extract and; group 6: *P. berghei* + 600 mg/kg body weight bark extract

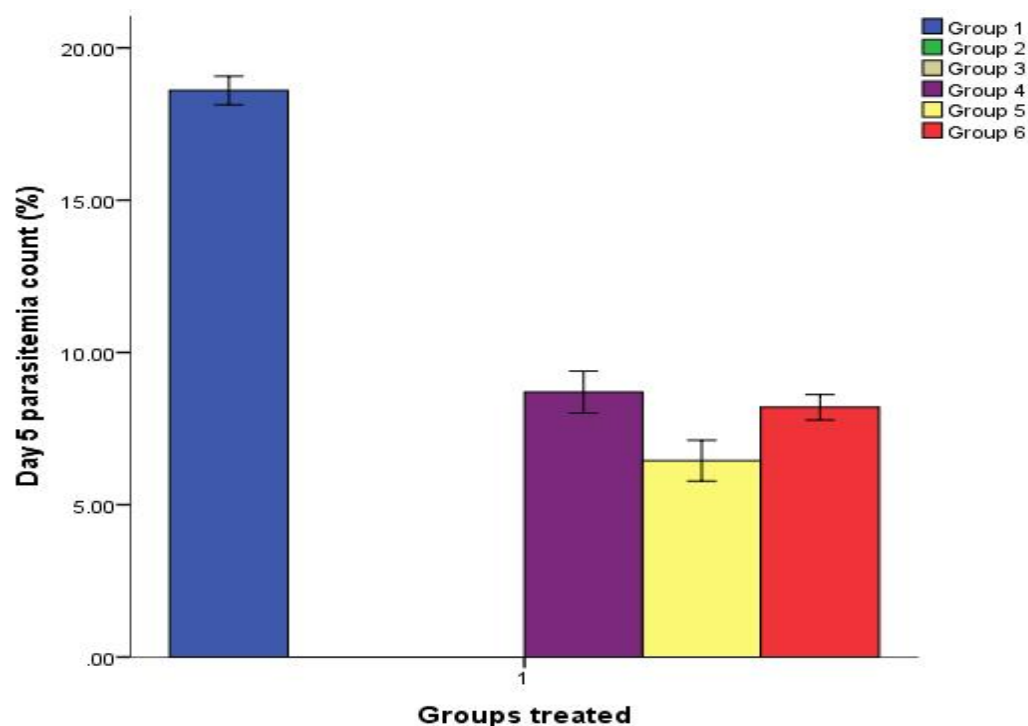


Figure 6: Parasitemia Count (%) for Day 5

Group 1: *P. berghei* + 0.2 ml normal saline, group 2: *P. berghei* + 5 mg/kg body weight Chloroquine, group 3: 0. 2 ml normal saline, group 4: *P. berghei* + 200 mg/kg body weight bark extract, group 5: *P. berghei* + 400 mg/kg body weight bark extract and; group 6: *P. berghei* + 600 mg/kg body weight bark extract

4.0 DISCUSSION.

This study revealed that bark extract of *K. grandifoliola* contains bioactive compounds such as alkaloids, saponins, flavonoids, tannins and glycosides, this agrees with Tor-Anyiin (7).

The outcome of the acute toxicity test in this study revealed no observable behavioral signs of toxicity and mortality suggest that the ethanolic extract of *K. grandifoliola* may not be toxic to the experimental mice at this dosage used and therefore be considered to be safe for consumption in treatment of malaria fever and other ailments.

The decreased in the body weight of both the infected and untreated mice (negative control) agrees with Basir [8]. that weight decreased in the infected mice with *Plasmodium berghei* might be attributed to the occurrence of anorexia, that is loss of appetite which is usually associated with malaria infection

The dose dependent antiparasmodial activity showed in this study corroborates with Ginsburg and Deharo and Ginsburg [9] who observed parasites clearing with ethanolic extract of medicinal plants such as *K. grandifoliola* as attributed to the presence of phytochemical constituent like Alkaloids and others in the studied extract.

4.1 CONCLUSION

The results obtained from this study revealed that the ethanolic bark extract of the *Khaya grandifoliola* significantly $P<0.05$ produced low parasitemia and improved in *Plasmodium berghei* infected mice and can be used in the treatment of malaria. Also, the bark extract is capable of reducing parasitaemia in the infected experimented mice.

ETHICAL APPROVAL

The whole experimental management, handling and care were approved by the Research and Ethics Committee of Microbiology Department School of Science, The Federal University of Technology, Akure, Nigeria.

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