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# *In vivo* Evaluation of the Antiplasmodial Activity of *Sterculia setigera* Leaf Extract on *Plasmodium berghei* Infected Mice

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## ABSTRACT

The developing resistance of malaria parasite to currently available anti-malarial drugs lead to search for novel effective drugs. *Sterculia setigera* a deciduous tree, commonly known as Karaya gum tree in Northern Nigeria. Traditionally, its boiled leaves have been used in the treatment of malaria. The present study aims investigate the toxicological and *in vivo* antiplasmodial effects of *S. setigera* extract on albino mice infected with *Plasmodium berghei*. Swiss albino mice (15-25g), chloroquine sensitive *P. berghei* and Fresh *S. setigera* leaves were collected, air dried, ethanol extract obtained and used for the study. The modified Lorke method was adopted to determine acute toxicity, with 3 groups of mice intraperitoneally administered varying doses of the extracts in 2 phases. The malaria parasite *plasmodium berghei* was inoculated into the apparently healthy Swiss albino mice. The curative evaluation of grouped mice used at various concentrations (150mg/kg, 300mg/kg, and 1200mg/kg) alongside negative (distilled water) and positive (10mg/kg chloroquine) controls were performed. The parameters of body weight, packed cell volume and parasitemia were determined using Ranes' and Peters test. The Phytochemical evaluation of the extract revealed the presence of flavonoids, tannins, steroids and triterpenes while, cardiac glycosides and anthraquiones were absent. The single oral dose of 5000mg/kg toxicity study was not found to be toxic after several observations. The crude extract in doses of 150mg/kg, 300mg/kg and 1200mg/kg administered to different group of mice showed decreased average parasitemia, while increased packed cell was observed. The crude extract also prevented weight loss on the last day of the treatment. The crude extract showed significant antiplasmodial activity against *P. berghei*, depending on the dosage. All extracts in treatment groups decrease parasitemia level in infected mice. This study suggests that the leaf extract of *S. setigera* possess antiplasmodial activity against *plasmodium berghei* in a dose dependent manner, and prevented loss of weight and increase packed cell volume which. establish a scientific justification on the traditional use of *S. setigera* in management of malaria.

**Keywords:** Antiplasmodial Activity, *Sterculia setigera*, *Plasmodium Berghei*, Leaf Extract,

## INTRODUCTION

Malaria is a mosquito-borne disease of humans and other animals, and a disease caused by eukaryotic protists of the genus *plasmodium.* Malaria remains a critical problem in global public health (Dokunmu *et al*., 2019) and continues to remain among the top three infectious diseases (malaria, tuberculosis and HIV). It remains a public health challenge globally (Mboowa, 2014). Despite advancements in antimalarial treatment, resistance to existing drugs is a significant problem that continues to pose threat treatment and has resulted in unacceptably high levels of disease and death (WHO, 2020). In keeping with the global priority to reduce the burden of the disease, and of malaria eradication (WHO guidelines for malaria, 2021), the challenge of malaria has necessitated a continuous search for new

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and effective antimalarial agents with novel mechanism of action.

Traditionally, plants have been a valuable resource in this search, given their historical use and medicinal potentials. *S. setigera* is a plant native to tropical regions of Africa has been used to treat various ailments; part of this tree, especially its leaves have been utilized in prophylactic, diagnosis, enhancement, or treatment of physical/mental illness (WHO,2018). However, scientific validation and detailed assessment of its efficacy against malaria are still lacking. The characterization of it potentials could give a better understanding of its mechanism of action and may lead to discovery of novel antimalarial compounds paving way for development of new drugs. This study aims to investigate the antiplasmodial activity of *S. setigera* leaf extract on *P. gerghei*-infected mice.

## MATERIALS AND METHODS

### Mice and Plant Source

Swiss albino mice (15-25g) and chloroquine sensitive *P. berghei* used for the study were obtained from the animal house of the Department of Pharmacy, and Pharmacology and Therapeutic respectively in Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria, Kaduna state, Nigeria. The mice were bred (maintained on standard commercial diets and water given *ad libtium*) to acclimatize for 7 days prior to their randomized grouping into the experimental groups.

### Collection, Identification and Processing *S. setigera* Leaf

The plant *S. setigera* was identified in the Herbarium unit, Department of Botany, Ahmadu Bello University, Zaria

### Plant Identification and Processing of Leaves

Fresh leaves of *S. setigera* were sourced and collected around Goruba village in Samaru District of Sabon gari Local Government Area, Kaduna State, and taken for proper identification and authentication at the Department of Botany, Ahmadu Bello University, Zaria, where a voucher specimen number ABU0950212 was assigned... *S. setigera* leaves were air-dried at room temperature and weighed periodically to a constant weight was obtained. The dried leaves were then pulverized to coarse powder using a dry mechanical mill. The powder was packed in a tight container and stored a cool dry place.

### Extraction of *S. setigera* Leaf

The extraction process yielded an extract of 18.05g. the percentage yield of was calculated using the formula below, and the yield was found to be 4 %.

### Extraction of Bioactive Compounds

The extraction of *S. setigera* leaves was carried out by the use of soxhlet apparatus; 70g of the powder leaves was weighed. 600ml of ethanol was transferred into a round bottom flask another 200ml was transferred into the condenser. The soxhlet apparatus was set and allowed to run for 16-24 hours after which the ethanol extract was collected and put in a water bath to evaporate at 64°C to dry. The dried extract was weighed and transferred into a sample container and kept in a refrigerator for subsequent use. The percentage yield determined (Ihekwereme *et al*., 2016).

### Phytochemical Screening

The phytochemical screening of S. setigera leaves was carried out using standard methods described by (Harborne, 2009; Evans, 2009).

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***Test for Cardiac Glycosides***

Keller-Killiani's Test: 0.5 g ethanolic extract of the leaf of *S. setigera* extract was dissolved in glacial acetic acid containing ferric chloride and one drop of sulphuric acid was added to the solution. The appearance of reddish-brown colouration at the interphase indicated the presence of deoxy-sugar.

***Test for flavonoids***

Sodium Hydroxide Test: 0.5 g of ethanolic extract of the leaf of *S. setigera* was dissolved in water and filtered. 2 ml of 10% aqueous sodium hydroxide solution was then added. The solution was observed for the presence of yellow colour. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was used as an indication for the presence of flavonoids.

***Test for anthraquinones***

Bontrager's Test: To 0.5 g of ethanolic extract of the leaf of *S. setigera*, 10 ml of chloroform was added and shaken. This was then filtered and 5 ml of 10% ammonia solution was added to the filtrate. The presence of pink or cherry red colour in the lower layer indicated the presence of combined anthracene or anthraquinone derivatives.

***Test for steroids and triterpenes***

Liebermann-Burchard Test: 1ml of acetic anhydride was added to 0.5 g of ethanolic extract of the leaf of *S. setigera*, it was dissolved in 1ml of chloroform. Concentrated sulphuric acid was then added gently by the side of the test tube to form lower layer at the junction of the two liquids. Formation of reddish brown or violet brown ring, the upper layer bluish green or violet indicated the presence of sterols and or triterpenes.

***Test for tannins***

Ferric Chloride Test: 0.5 g of ethanolic leaf extract was dissolved in 5 ml of water each and filtered. 2 drops of ferric chloride solution were added to the filtrate. Appearance of blue-black or green or blue-green (condensed tannins) precipitate indicated the presence of tannins.

**Acute Toxicity of the Extract**

The modified Lorke method was adopted to determine the $LD_{50}$ of the crude extract of *S. setigera*. The was performed using male mice. They were subjected to fasting overnight and divided into three groups of 3 mice per cage. In the first phase, the mice received 10, 100, and 1000mg/kg of the extract intraperitoneally, and closely observed for the first 6-24hours for signs of toxicity and mortality. For the second phase, the 3 groups of mice per cage received 1600, 3200, and 4000mg/kg of the extract intraperitoneally, and observed for signs of toxicity and mortality at regular intervals for 24hours, 48hours, and 72hours (Ihekwereme *et al*., 2016).

**Experimental Design**

The mice were grouped into five (5) groups of three (3) as follows: Group 1: Positive control group treated with 10mg/kg chloroquine **(PC),** Group 2: Negative control group treated with distilled water **(NC),** Group 3: Infected group treated with 150mg/kg body weight of *S. setigera* crude extract **(IT150MI),** Group 4: Infected group treated with 300mg/kg body weight of *S. setigera* crude extract **(IT300MI),** Group 5: Infected group treated with 1200mg/kg body weight of *S. setigera* crude extract **(IT1200MI).**

**Inoculation of Experimental Animals**

Blood was intraocularly collected via punch from two donor mice with rising parasitaemia of 25% and 27% in heparinized syringes. Standard inoculums of $1\times10^7$ *P. berghei*

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infected erythrocyte in 0.2 ml were prepared by diluting infected blood with 0.9% normal saline. Each mouse was intra-peritoneally injection with the blood suspension (0.2ml) containing $1\times10^7$ parasitized erythrocytes.(Okokon *et al.*, 2017).

**Curative Test**

This evaluation was performed according to the method described by (Ryley and Peters, 1970). 20 mice were used for curative experiment. Infected animals were divided into 5 groups (n = 5) of 4 mice each. The infected mice were kept for 72hours for their body to pick up the parasite. Two control groups were used namely, positive (infected and treated with 10mg/kg of chloroquine), and negative (infected and treated with distilled water). Three different concentrations of 150mg/kg, 300mg/kg, and 1200mg/kg body weight of *S. setigera* extract was administered to groups 3-5 respectively. All treatments were administered orally using feeding cannula and the treatment lasted for 4 consecutive days. Blood samples were collected from the tip of the tails of the animals on each day to check for the parasitaemia level.

**Estimation of Parasitaemia**

A small drop of blood was collected from the tail of each of the animals in each group, using clean, non-greasy slides to make thin blood smears and allowed to air-dry. The thin films were then fixed using few drops of methanol. The films were left for about 15-20 minutes to air-dry. Thereafter they were washed off and stained with Giemsa stain for 45 minutes. The stain was washed off and slides were left to air-dry, before they were viewed under light microscope using oil immersion objective. Each of the blood films prepared was mounted and viewed under the microscope using the ×100 objective. The number of parasitized red blood cells that were seen per film was counted and recorded. The average percentage parasitaemia was calculated using the formula (Bantie *et al*., 2014)

$$\% \text{ parasitaemia} = \frac{\text{Total number of parasitized erythrocytes}}{\text{Total number of erythrocytes counted}} \times 100$$

**Determination of Packed Cell Volume (PCV)**

The tip of the tail of each mouse was cut using a sterilized scissors. Blood was collected by gently milking the tail using heparined microhaematocrit capillary tubes and sealed at their dry end with flame. The tubes were then placed in a microhaematocrit centrifuge with the sealed end outwards. The blood was centrifuged at 12000rpm for 5min. the packed cell volume was determined using the following relation (Bantie *et al*., 2014)

$$\text{PCV} = \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}} \times 100$$

**Monitoring of Body Weight Changes**

For the curative test, body weight of each mouse was measured before inoculation, 3 days after inoculation, and all the treatment days using a sensitive digital weighing scale. In order to rule out the effect of the extract on body weight, and PCV; the crude extract was administered to healthy mice at the doses used for four days treatment.

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### Data Analysis

Data obtained were expressed as mean ± standard deviation (SD). Data were analyzed using one way analysis of variance (ANOVA) with the help of statistical package for social science (SPSS) version 23 for window. Duncan post hoc test was done to compare the differences among the mean of the various treatment groups. P. value less than 0.05 ($p$<0.05) was considered statistically significant.

## RESULTS

### Phytochemical Screening

The extract was subjected to basic phytochemical screening using standard methods described by (Harborne, 2009; Evans, 2009) and the result on the Table 1 below was obtain.

**Table 1.** Result of phytochemical screening of *s. setigera* leaf extract

| | Phytochemical constituent | |
|---|---|---|
| **1** | Flavonoids | + |
| **2** | Triterpenes | + |
| **3** | Cardiac glycosides | + |
| **4** | Tannins | + |
| **5** | Anthraquiones | + |
| **6** | Steroids | - |

KEYS : Present (+), Absent (-)

### Test of toxicity studies of *S. setigera* leaf

The acute toxicity study was conducted according Lorke method. The limit dose showed no signs and symptoms of toxicity at 5000 mg/kg body weight (Table 2).

**Table 2:** Result for toxicity study of *S. setigera* Crude extract

| Groups | Dose (mg/kg) | Mortality |
|---|---|---|
| Group 1 | 1500 | 0/3 |
| Group 2 | 2500 | 0/3 |
| Group 3 | 3500 | 0/3 |
| Group 4 | 5000 | 0/3 |

### Effects of the Crude Extract on the Body Weight of *P. berghei* Infected Mice.

There is significant increase in the positive control group and all treatment groups, while there is significant decrease in the negative control group as shown by the chart below.

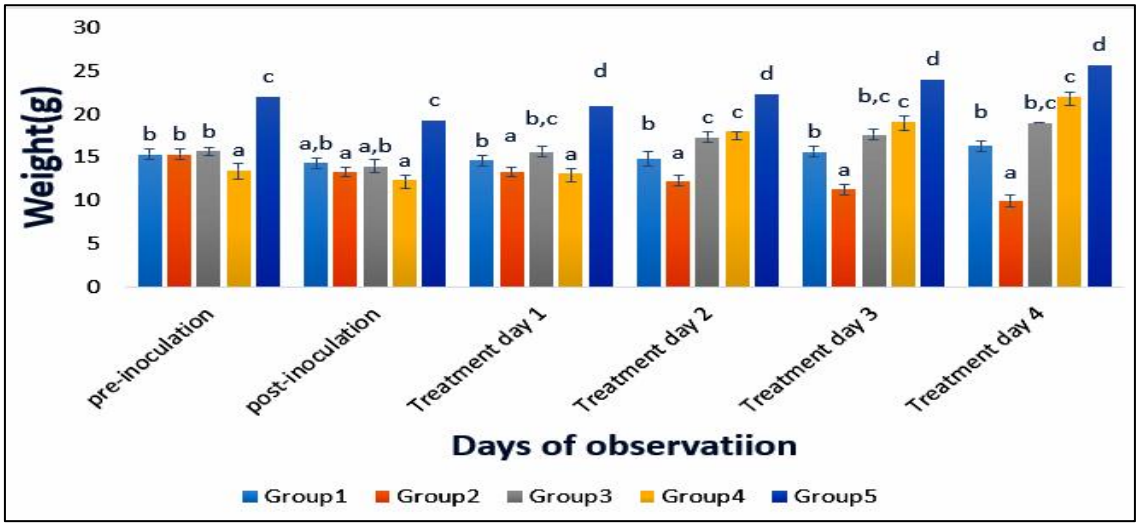


**Figure 1:** Effects of *S. setigera* leaf extract on body weight of *P. berghei* infected mice.

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Values are presented as mean of triplicate ± standard deviation. Different Superscript differ significantly per day at p±0.05. Keys: Group 1 = Positive control, Group 2= Negative control, Group 3 = Treatment group 1, Group 4 = Treatment group 2, Group 5= Treatment group 3.

### Effects of the Leaf Extract on Packed Cell Volume of *P. Berghei* Infected Mice

The leaf extract was able to increase packed cell volume all treatment groups while there is significant decrease in negative control as shown in Figure 2.

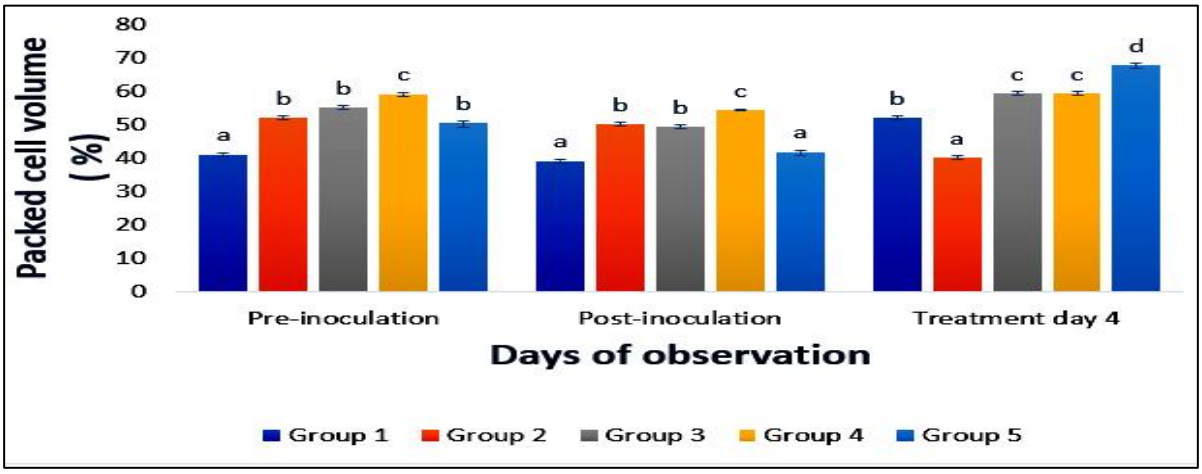


**Figure 2:** Effects of *S. setigera* leaf extract on PCV of *P. berghei* infected mice.

Values are presented as mean of triplicates ± standard deviation. Different Superscript differ significantly per day ($p \leq 0.05$). Keys: Group 1 = Positive control, Group 2 =Negative control, Group 3 = Treatment group 1, Group 4 =Treatment group 2, Group 5 = Treatment group 3.

### Effects of the Extract on Parasitemia of *P. berghei* Infected Mice

There is significant decrease in parasitemia in all treated group and positive control group indicating the effectiveness of the extract and standard drug in decreasing parasitemia. The significant increase seen in group two was due to the fact that distilled water was used in treating group two which serve as the negative control (Figure 3).

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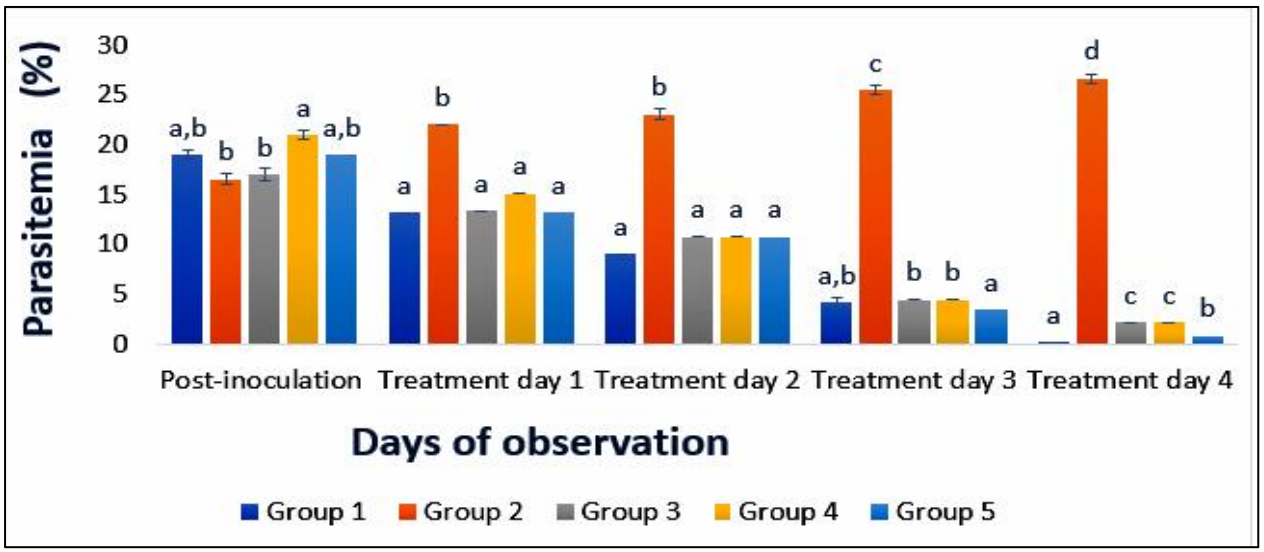


**Figure 3:** Effects of *S. setigera* leaf extract on Parasitemia of *P. berghei* infected mice.

Values are presented as mean of triplicates ± standard deviation. Different Superscript differ significantly per day at $p \leq 0.05$. Keys: Group 1= Positive control, Group 2= Negative control, Group 3= Treatment group 1, Group 4= Treatment group 2, Group 5= Treatment group 3

## DISCUSSION

*P. berghei* is used in the induction of malaria because it has a very similar life cycle to the species that infect humans, and it causes diseases in mice which have similar signs to those seen in humans (Mojarrab *et al*., 2014). Chemotherapy is threatened due to development of resistance, (Sergi *et.al*., 2019) thereby leading to the need of new, effective, safe and affordable anti-malarial drugs. Many of the currently available antimalarial drugs have been developed from plant and natural product such as quinine.*S setigera* is traditionally used for the treatment of various ailments including malaria (Abubakar *et al*., 2015; Deshpande and Bhalsing, 2015). This research aimed at providing a scientific basis for its' traditionally used as antimalarial drug, revealed the following; The result of extraction using soxhlet apparatus gave an extract of 18.05g with percentage yield of 4 %. The qualitative phytochemical screening of the crude revealed the presence of flavonoids, Saponins, tannins, steroids and triterpenes, and absence of Anthraquiones, and cardiac glycosides similar to Adelaku *et al*., 2014 and Babalola *et al*., 2012.

The acute toxicity profile of the crude extract at an oral dose at 5000 mg/kg was found to be practically non-toxic to mice after several observations of the mice. Body weight loss, anaemia and body temperature reduction are the general features of malaria – infected mice (Prapaporn *et al.,* 2019). A good anti-malarial agent obtained from plants are expected to prevent body weight loss in infected mice due to the rise in parasitemia (Bantie *et al.,* 2014; Kevin et al., 2020). There was no body weight loss of *P. berghei* infected mice treated with the crude extract (table 3) therefore, the extract and standard drug prevented weight loss associated with increase in parasitemia level. The parasitemia decrease significantly in positive control and all treatment group, indicating the antimalarial potential of the extract, while there is significant increase in the negative control group.

The PCV was measured to evaluate the effectiveness of the extract and in preventing

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haemolysis due to escalating parasitemia level (Bantie *et al.,* 2014). The cause of anaemia during malaria is the destruction of infected RBCs by the parasite. The result showed that at the doses of 150, 300 and 1200 mg/kg showed that the PCV dropped after inoculation which is expected due to multiplication of parasitemia without treatment according to Bantie *et al.,* (2014), while at day 7 the PCV increased. This was a clear indication that the extract and chloroquine were effective in preventing haemolysis due to escalating parasitemia level (Bantie *et al.,* 2014). The results of the *curative test* (Ranes' test) showed that the extract at all doses have antimalarial activity because it counteracts all the symptoms of malaria seen in the infected mice. The extract prevented weight loss of *P. berghei* infected mice when treated with the extract and chloroquine but significant weight loss was seen the *P. berghei* infected mice in negative control group. The extract and the standard drug were able to reverse the PCV reduction due to multiplication of *P. berghei* parasites in infected mice. The study revealed that *S. setigera* leaves extract possess good antiplasmodial activity.

## CONCLUSION

This study suggests that the leaf extract of *S. setigera* possess antiplasmodial activity against *plasmodium berghei* in a dose dependent manner, and prevented loss of weight and increase packed cell volume which. establish a scientific justification on the traditional use of *S. setigera* in management of malaria.

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