



Antiplasmodial Efficacy of Stem Bark Extracts of *Pseudocedrela kotschy* in Mice Infected with *Plasmodium berghei berghei*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AD and DPY designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript, read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To evaluate the antiplasmodial efficacy of stem bark extracts of *P. kotschy* against *P. berghei berghei* in mice.

Study Design: Extraction and administration of plant extracts and evaluation of daily parasitaemia of infected mice.

Place and Duration of Study: Department of Pharmacognosy; Animal House. Department of Pharmacology and Department of Zoology, University of Jos, Jos, Nigeria, between June, 2008 and November, 2011.

Methodology: Dry zone cedar (*Pseudocedrela kotschy*) Family: Meliaceae, stem bark powder was successively extracted using ethyl acetate, ethanol and aqueous solvents. A total of one hundred and twenty mice for each extract (ethanol, ethyl acetate and aqueous) were divided into three groups of forty mice per each test (suppressive, curative and prophylactic). They were inoculated with drug sensitive NK 65 *Plasmodium berghei berghei*. In each test animals were divided into five groups, each consisted of eight animals and treated separately with one of the following: 50, 100, and 200 mg/kg extracts, chloroquine / pyrimethamine and normal saline. Blood films were prepared and examined, and the changes in percentage parasitaemia were evaluated.

Results: The ethanol, ethyl acetate and aqueous crude extracts of *P. kotschy* at 200 mg/kg significantly ($P=0.05$) inhibited the parasitaemia by 39.43%, 26.99% and 28.36% respectively in the suppressive test. Ethanol and ethyl acetate crude extracts also showed

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significant ($p=0.05$) cure rate of 29.17 % and 20.28 % respectively. However there was no significant ($p>0.05$) reduction in parasitaemia load in the prophylactic tests.

Conclusion: The results of the study showed that *P. kotschyi* stem bark indeed has antiplasmodial property.

Keywords: Antiplasmodial efficacy; *P. kotschyi*; *P. berghei berghei*; parasitaemia; mice.

1. INTRODUCTION

Globally millions of deaths attributed to malaria are being recorded. There were estimated 247 million malaria cases among 3.3 billion (half of the world's population) people at risk in 2006, causing nearly a million deaths, of which 90% were in African region and 4% in each of the South East Asia and Eastern Mediterranean regions [1]. With massive increases in the delivery of interventions, the estimated annual number of malaria deaths were 781,000 (0.78 million) in 2009 [2].

The economic burden of malaria illness on Africa is huge. One in six child deaths in Africa is due to malaria and as much as 40% of health care spending in the worst affected countries goes on treatment, costing the continent \$12 billion per year [3,4].

A major problem in the treatment of malaria is that of drug resistance. Resistance of *P. falciparum* to chloroquine and other anti-malarials, including the relatively recently introduced mefloquine, is reported in different places [5,6]. Other species of *Plasmodium* also show resistance to some of these drugs. Another issue is the continuous use of counterfeit drugs and single-based therapy which evolved in drug resistance. As at November 2010, twenty five countries (25), mostly in Africa, were still allowing the marketing of artemisinin-based monotherapies, and thirty nine (39) pharmaceutical companies, mostly in India, were manufacturing these products [7].

Plants form major part of treatments used by traditional healers in many societies. There are several plants, which have long been utilized by traditional practitioners for curing diseases and some of these plants are still used today for medical purposes. Thus, many plants have acquired reputation for being useful against malaria [8]. Ethno-medicines play a central role in the search for and development of new drugs [9,10]. [11] In their ethno-graphic evaluation of ethnomedicine in Nigeria reported that many herbal remedies have been used traditionally for treatment of febrile illnesses in South-Western Nigeria. In categorizing febrile illnesses, alongside the causes, and symptoms, ordinary fever and rainy season fever are the two illnesses symptomatically related to malaria infection. Studies have documented over 1,200 plant species from 160 families used in the treatment of malaria or fever [12].

Pseudocedrela kotschyi (Plate 1) is Savanna woodland plant, chiefly of the Guinea zone on moister heavy soils of valleys. The plant when well grown resembles *Khaya senegalensis* and is often found growing with it and *Daniellia oliveri*. When well grown, it is one of the largest trees of the Savanna, but it suffers from fires, and crooked stems. Trees are commonly 20-30 feet high but up to 60 feet high and six feet girth on good sites. Large girths are more frequently seen than great heights. The crown is rounded with ascending branches. The bark is thick silvery-grey and fairly regularly fissured into small square pieces. The tree reproduces from root-suckers, the seeds being often destroyed by bush fires [13,14].



A. Crown of leaves

B. Stem part

Plate 1. *Pseudocedrela kotschy* plant

Pseudocedrela kotschy Harm (Meliaceae) is used for various purposes. The low to none antimicrobial activity of *P.kotschy* against microorganisms: *Staphylococcus* spp, *Streptococcus* spp, *Candida* spp, *Bacillus* spp, *Cladosporium* spp and *Pseudomonas* spp was reported [15,16,17]. Kassim et al. [18] reported the inhibition of *Plasmodium falciparum* trophozoites transformation into schizonts by *P. kotschy* root extract.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The plant *Pseudocedrela kotschy* (Meliaceae), used for the study was obtained from Tim along Mangu – Bwai road in Mangu Local Government area and identified by taxonomists from the Federal College of Forestry, Jos and Department of Plant Science and Technology, University of Jos, Jos. A voucher specimen was deposited at The Federal College of Forestry, Jos and in the Department of Pharmacognosy, University of Jos, Jos with specimen numbers SF00108J and UJ/PGNS/HSP/0901 respectively. The stem parts of the plants above the soil were cut using a knife. The stem were peeled and dried under shade as described by [19]. The dried materials were ground to powder using mortar and pestle.

2.2 Extraction of Plant Materials

This stem powder was successively (serially) extracted in a similar method described by [20, 21] using hexane, ethyl acetate, methanol, ethanol and then water in that order of increasing solvent polarity. The results of previous phytochemical screening led to the choice of ethyl

acetate, ethanol and aqueous extracts used. 120 grammes of the powdered stem bark was measured and transferred into a flask and 2.5 liters of each solvent was added and capped with the cover in each step to macerate for 24 hours with intermittent agitations. The marc was always removed and dried to evaporate the solvents before re-introducing into the container for each subsequent extraction. The extracts were collected in a flask and concentrated to dryness using a rotary evaporator and water bath at 40°C–60°C. The percentage yield for each sample was determined and crude extracts were stored in a refrigerator at 4°C for further use.

2.3 Experimental Animals / Parasites

Four weeks old male and female albino mice, free from contaminating organisms, weighing 18-23g used in the study, were obtained from the animal house, University of Jos. They were fed on a standard rodent's diet consisted of crude protein, fat, calcium, available phosphate, vitamins, crude fibre and constant supply of water, in plastic cages measuring 25cm x20cm x10cm with a metal cover for free passage of air, at room temperature of about 27°C. Chloroquine sensitive *Plasmodium berghei berghei* NK 65 was used to test the plant extracts. The parasites were obtained from the National Institute for Pharmaceutical Research and Development (NIPRD) Idu-Abuja and the Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria by inoculation of uninfected mice with blood from *P. berghei* infected mice. The parasites were maintained by successive inoculations of parasite free mice every four or five days with 0.2 ml of the diluted blood from the infected mice using one (1) ml syringe and needle.

2.4 Antiplasmodial Studies

2.4.1 Suppressive test

The suppressive test was conducted in a similar method used by [22,23]. 40 mice were divided into 5 groups of 8 animals each. Three of the 5 groups were each administered one of the following: 50 mg/kg, 100 mg/kg or 200 mg/kg of the extract. The choice of the doses was from the results of acute toxicity test. One of the remaining groups (Control 1) was administered 0.2ml normal saline while the other group (Control 2) received chloroquine phosphate (5 mg/kg). Each mouse was inoculated on the first day (Do), intraperitoneally, with infected blood containing *Plasmodium berghei berghei* parasitized red blood cells. The mice were treated daily from day 0 (immediately after infection) to day 3. Thin smears fixed in methanol stained with Giemsa stain were prepared from the tail blood of each mouse daily from day 4 to day 7, examined microscopically under oil immersion and the percentage parasitaemia was determined by counting the parasitized erythrocytes in at least ten random fields. The mean parasite count for each group was determined and the average percentage chemosuppression for each dose was calculated as $[(A-B)/A]$, where A is the average percentage of parasitaemia in the normal saline group (control 1) and B is the average percentage of parasitaemia in the test groups.

2.4.2 Curative or rane test

This was conducted in a similar method adopted by [19,23]. Another set of 40 albino mice were infected with parasitized erythrocytes. Seventy-two hours after infection, the mice were divided into 5 groups of 8 animals each. Three of the 5 groups was each administered one of the following: 50 mg/kg, 100 mg/kg or 200 mg/kg of the extract. The fourth group (control 1)

was given 0.2ml normal saline while the last group (control 2) received 5 mg/kg of chloroquine phosphate. Each mouse was treated orally once daily with the dose for 5 consecutive days (D4-D8) post inoculation during which the parasitaemia level was monitored daily.

2.4.3 Prophylactic or repository test

This was carried out according to the method of [24]. The mice were divided into 5 groups of 8 animals each. Three of the 5 groups were each administered one of the following: 50 mg/kg, 100 mg/kg or 200 mg/kg of the extract. The fourth group (control 1) was given 0.2ml normal saline while the last group (control 2) received 1.2 mg/kg of pyrimethamine for four consecutive days. On day four the mice were inoculated with *P. berghei berghei*. Seventy two hours later (D3), smears were made from the mice (D3-D7) and the parasitaemia levels assessed.

2.4.4 Statistical analysis

Data were analysed using one way analysis of variance (ANOVA) to compare the parasitaemia of mice between doses and among treatment and control (normal saline) group.

3. RESULTS

The percentage yields of crude extracts of *P. kotschy* stem bark after evaporation was higher in ethanol 20.4g (17%) followed by methanol, water, ethyl acetate and hexane extracts which gave 15.6 (13%), 10.2g (8.5%), 1.92g (1.6%) and 0.52 (0.42%) respectively. The mean percentage parasitaemia in mice treated with extracts increased continuously through the experimental period from day three or day four to day seven or eight for suppressive or curative and prophylactic test respectively in groups treated with the extracts and normal saline (control), While those given chloroquine/pyrimethamine decreased towards zero percent.

3.1 Ethanol Extracts Effects

The suppressive test (Fig. 1) showed a sudden increase in parasitaemia from 7 ± 0.54 and 2.5 ± 0.38 in the normal saline control 1 and 200 mg/kg groups respectively on day four to 12.86 ± 1.86 and 9.5 ± 1.05 respectively on day five, thereafter a gradual increase to day seven in groups treated with 50 mg/kg, 100 mg/kg and 200 mg/kg ethanol extract and another sharp increase was observed on day eight. Although the normal saline control group showed a continuous increase resulting to parasitaemia of 40 ± 2.08 on day eight, the parasitaemia in the chloroquine control 2 group decreased continuously from 2.5 ± 0.27 to 0 on days four and eight respectively. Generally the groups treated with extract and standard drug had significantly ($p = .05$ and $p = .001$ respectively) lower parasitaemia compared with the normal saline control 1 group. The parasitaemia in all the mice treated with the plant extract in the curative test increased continuous from day three to day four except the group administered 200 mg/kg which had a gradual increase from 9.88 ± 0.77 to 16.75 ± 1.69 on day four to five respectively (Fig. 2). The extract treated groups had a lower parasitaemia than the untreated normal saline control 1 with 44.88 ± 2.35 and 45.63 ± 2.6 in the 50 mg/kg and the normal saline control 1 respectively on day seven. However there is a significant difference ($p = .05$) between the groups treated with ethanol extract and the control 1.

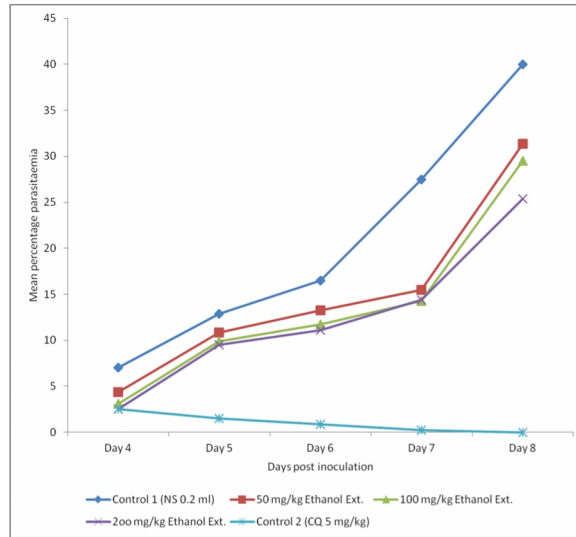


Fig. 1. Changes in parasitaemia in mice inoculated with *P.berghei berghei* on day 0 following daily oral treatment on day 0-3 with Ethanol Stem Bark Extract of *P. kotschyi* (SUPPRESSIVE TEST)
NS-Normal saline; CQ-Chloroquine ; Ext-Extract.

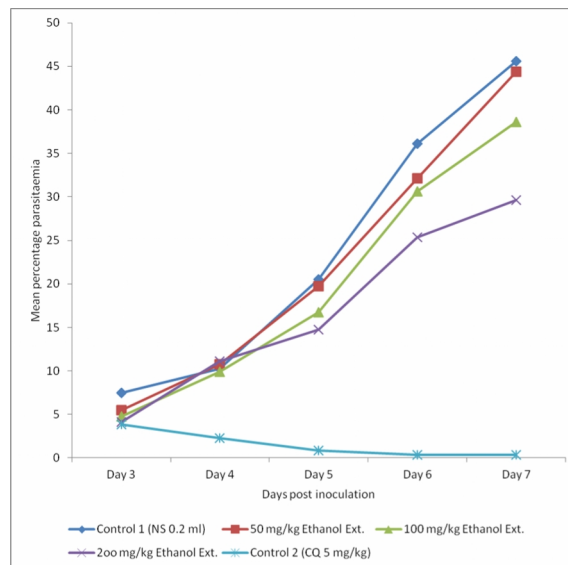


Fig. 2. Changes in parasitaemia in mice inoculated with *P.berghei berghei* on Day 0 treated orally on day 3-6 with ethanol stem bark extract of *P. kotschyi* (CURATIVE TEST)
NS- Normal saline; CQ-Chloroquine; Ext- Extract.

Fig. 3. shows the changes in parasitaemia in mice in the prophylactic treatment. The parasitaemia of the treated mice were slightly higher than 17.13 ± 1.92 and 25.63 ± 2.29

recorded in the normal saline control 1 group on day five and six respectively. The parasitaemia of normal saline on day seven was insignificantly ($p > .05$) higher 42.63 ± 3.33 than those of the extract treated groups.

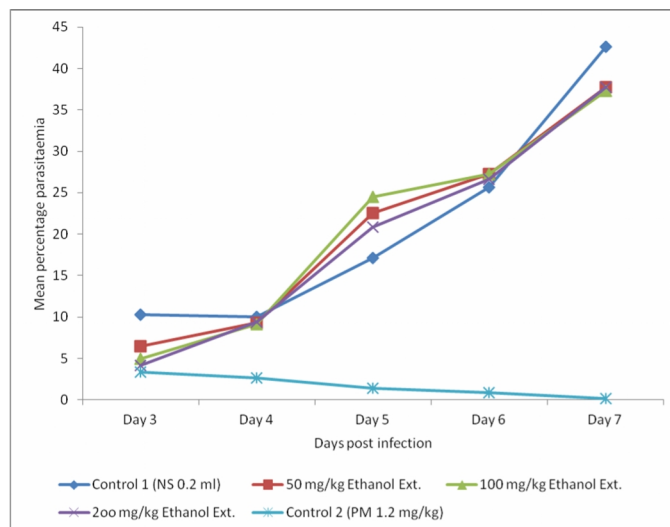


Fig. 3. Changes in parasitaemia in mice inoculated with *P.berghei berghei* (Day 0), 72 hours after four days of daily oral administration of ethanol stem bark extract of *P. kotschyi*. (PROPHYLAC TEST)
NS- Normal saline; PM- Pyrimethamine; Ext-Extract.

3.2 Aqueous Extract Effects

It can be observed in the suppressive test (Fig. 4) that there was steady increase in parasitaemia from 7.38 ± 0.82 on day four to 16.75 ± 1.24 on day six in the normal saline control 1 and to day seven in groups treated with the aqueous plant extract, thereafter a sharp increase was observed on day eight which had 41.75 ± 1.25 in the normal saline control group and 39.88 ± 2.05 , 37.75 ± 2.52 and 30.13 ± 1.57 in groups administered 50 mg/kg, 100 mg/kg and 200 mg/kg extract. There is a significant difference ($p = .05$) between extract groups and the control 1.

Fig. 5 shows the parasitaemia of aqueous extract in the curative test. There was a slight increase in parasitaemia from day four to day five and a sharp increase was observed from day five to day six in the normal saline control and untreated groups. On day seven almost similar parasitaemia were observed in the untreated control and group given 50 mg/kg which had 44.75 ± 2.81 and 44 ± 1.82 respectively. The other groups treated with the extract had insignificantly ($p > .05$) lower parasitaemia.

The prophylactic test as shown in Fig. 6 shows a continuous interlocking parasitaemia between the extract treated groups and the untreated control. The normal saline control 1 group and the group given 50 mg/kg extract had almost similar parasitaemia of 42.75 ± 2.91 and 42.5 ± 2.81 respectively on day seven and slightly lower than that in the other extract treated groups. The parasitaemia in all the groups administered the chloroquine/pyrimethamine standard drugs decreased continuously tending towards zero

percent. Analysis of variance showed that there is no significant difference ($p > .05$) between extract treated groups and the normal saline group.

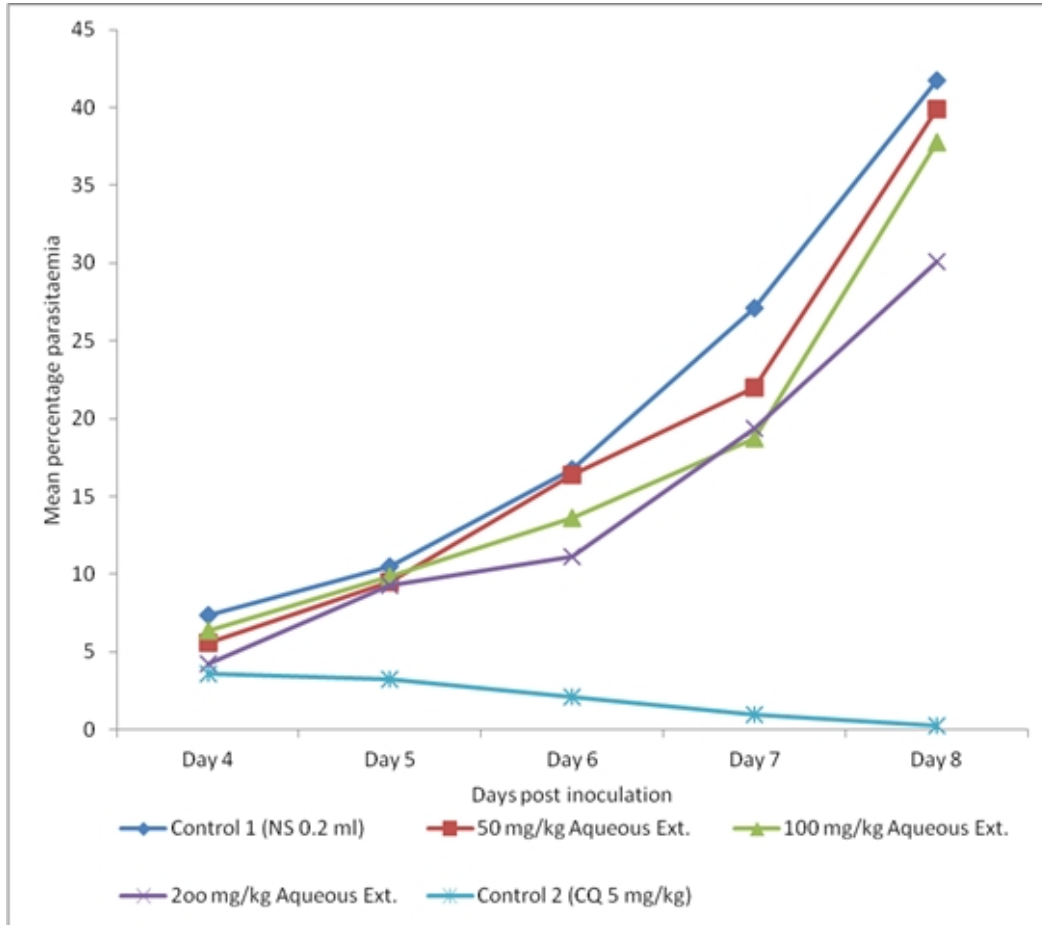


Fig. 4. Changes in parasitaemia in mice inoculated with *P.berghei berghei* on Day 0 following daily oral treatment on day 0-3 with aqueous stem bark extract of *P. kotschy* (SUPPRESSIVE TEST)
NS- Normal saline; CQ-Chloroquine; Ext-Extract.

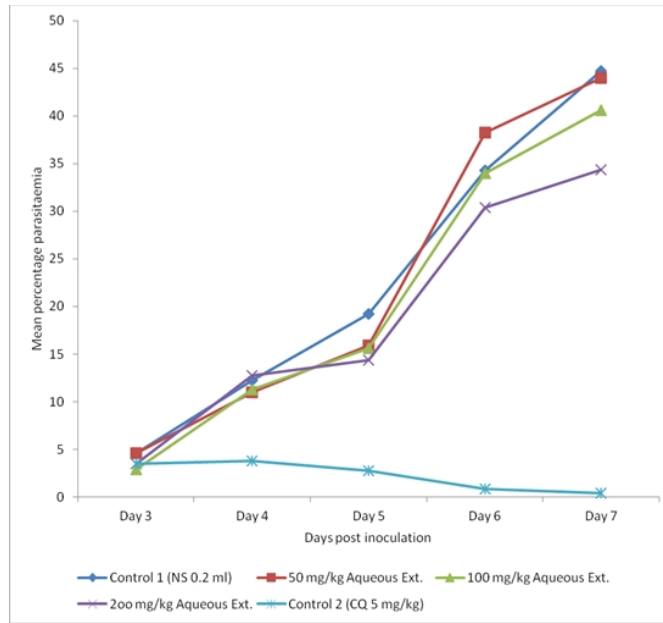


Fig. 5. Changes in parasitaemia in mice inoculated with *P.berghei berghei* on Day 0 treated orally on day 3-6 with aqueous stem bark extract of *P. kotschyi* (CURATIVE TEST)
 NS- Normal saline; CQ-Chloroquine ; Ext-Extract.

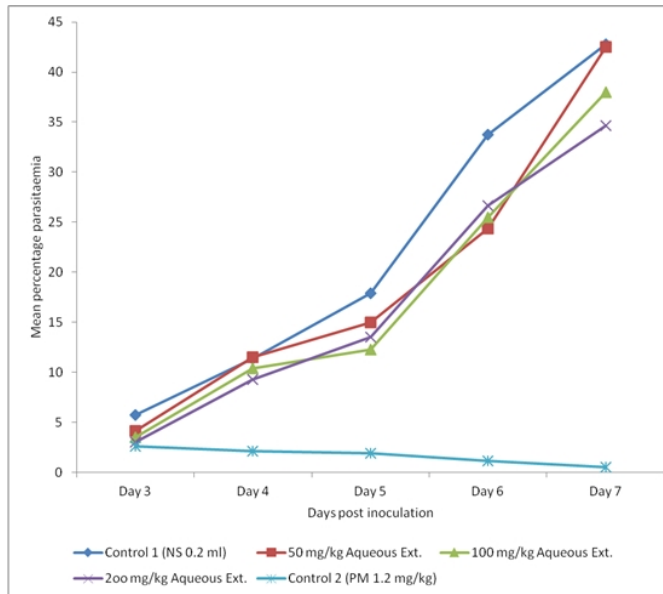


Fig. 6. Changes in parasitaemia in mice inoculated with *P.berghei berghei* (Day 0), 72 hours after four days of daily oral administration of aqueous stem bark extract of *P. kotschyi*. (PROPHYLACTIC TEST)
 NS- Normal saline; PM- Pyrimethamine; Ext-Extract.

3.3 Ethyl Acetate Extract Effect

Fig. 7 shows the suppressive effect with interlocking parasitaemia in the normal saline and extract treated groups from day four through day seven, after which lower parasitaemia was observed in the groups that received the extract than the normal saline control group on day seven and eight which had 38 ± 1.77 and 47.5 ± 1.52 in the normal saline and 31.25 ± 1.71 and 46.88 ± 1.22 in the group treated with 50 mg/kg on day seven and eight respectively. There is a significant ($p = .05$) decrease in the parasitaemia of mice treated with extract compared with the normal saline groups.

The change in parasitaemia in the curative test is shown in Fig. 8. There were nearly similar parasitaemia in all the extract treated and normal saline groups on day three and four the groups administered 50 mg/kg and 100 mg/kg had slightly higher parasitaemia than the normal saline control on day five but lower parasitaemia than the control on day six and seven. However the group administered 200 mg/kg had significantly ($p = .05$) lower parasitaemia than the normal saline control from day five to day seven with 23.5 ± 2.18 and 19.63 ± 1.48 on day four, 46.75 ± 1.40 and 32 ± 2.61 on day seven in the normal saline and 200 mg/kg groups respectively.

Fig. 9 shows the prophylactic effect of *P. kotschy* on parasitaemia in mice. There was interlocking effect with higher parasitaemia of 23.88 ± 1.85 at 50 and 100 mg/kg than 21.38 ± 1.13 in normal saline control on day five. The parasitaemia in groups administered 50 mg/kg were slightly higher (48.13 ± 1.74) than the 45.25 ± 1.31 observed in the normal saline control on day seven. The parasitaemia in group administered 200 mg/kg were insignificantly ($p > .05$) lower than the normal saline control 1 throughout the period of observation.

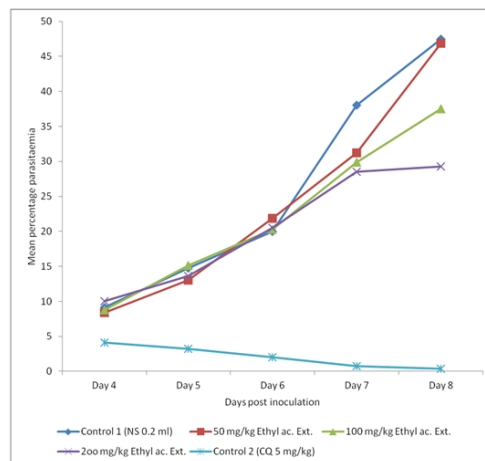


Fig. 7. Changes in Parasitaemia in Mice Inoculated with *P. berghei berghei* on Day 0, following daily oral treatment on day 0-3 with ethyl acetate stem bark extract of *P. kotschy* (SUPPRESSIVE TEST)
 NS- Normal saline; CQ-Chloroquine; Ext-Extract.

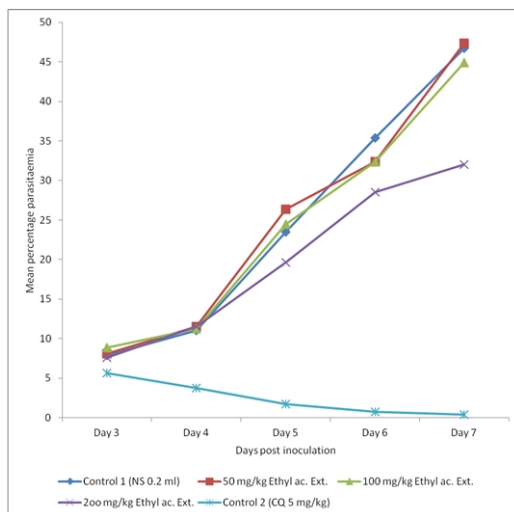


Fig. 8. Changes in parasitaemia in mice inoculated with *P. berghei berghei* on day 0. treated orally on day 3-6 with ethyl acetate stem bark extract of *P. kotschyi* (CURATIVE TEST)

NS- Normal Saline. CQ-Chloroquine. Ext-Extract.

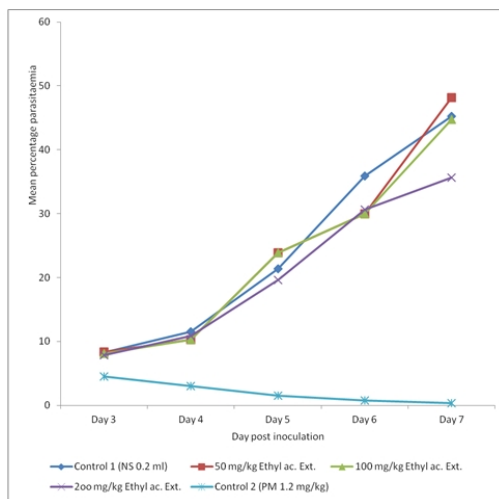


Fig. 9. Changes in parasitaemia in mice inoculated with *P. berghei berghei* (Day 0), 72 hours after four days of daily oral administration of ethyl acetate stem bark extract of *P. kotschyi*. (PROPHYLACTIC TEST)

NS- Normal saline. PM- Pyrimethamine. Ext-Extract.

4. DISCUSSION

The extract produced dose – dependent relationship, with the higher doses resulting in greater reduction in the parasite density/ load than the lower doses. The highest antiplasmodial suppression of 39.43 % observed in this study is low compared with 79.5% and 81.36% reported by [25,26] respectively. The difference in activity observed in this study

could be attributed to the differences in species of the plant and the periods of collection of the plant materials. The inhibition of *P. berghei* by *P. kotschy* extract could be attributed to the presence of anthraquinone, alkaloids, steroids phenols, tannins and saponins reported by [15,27]. These compounds inhibit the activity of enzyme cytochrome oxidase, of the malaria parasite thereby reducing the effectiveness of DNA to act as a template. This agrees with the finding reported by [28] on the activity of terpenoids against *P. falciparum*, *P. berghei*, *Trypanosom* epimastigotes, *Leishmania*, *Entamoeba* and *Giardia*. The result of this study is consistent with the findings of [28,29] that the quassinoids demonstrated antiplasmodial activity against *P. falciparum* and *P. berghei* by inhibiting protein synthesis in the parasite. [25,30,31] attributed the inhibition of *P. falciparum* and *P. berghei*, *in vitro* and *in vivo* to the activity of steroids, terpenoids, flavonoids and alkaloids. Limonene is a monoterpened found in essential oils of *Citrus* and other plants. [32] showed that parasite development was arrested and isoprenylation of proteins in *P. falciparum* was caused by the activity of limonene. The study revealed that the ethanolic extract of the plant reduced parasitaemia by 39.43 % when tested at an oral dose of 200 mg/kg, indicating that the effective dose of each extract were greater than 200 mg/kg. Reduction in parasitaemia was more evident in the groups administered with ethanol extract than in those tested with the aqueous and ethyl acetate extracts.

5. CONCLUSION

The present study is a comprehensive assessment of the effectiveness of dry zone cedar (*Pseudocedrela kotschy*) plant which is used locally against fever, in the treatment of malaria in mice. While all the three solvents crude extracts of *P. kotschy* demonstrated a significant ($P < 0.05$) suppressive effects in tests carried out and ethanol and ethyl acetate showed significant curative effects, there was no significant reduction in parasitaemia load in the prophylactic tests conducted indicating that the plant is probably not a potential prophylactic subject. The results of this study shows that the plant has antiplasmodial property but the antiplasmodial efficacy is not high, probably due to the doses used, so these findings scientifically prove the local belief that the plant is used for the treatment of fever.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTEREST

Authors do not have any competing interests.

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