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Research Article
Supressive and Prophylactic Potentials of Flavonoid-rich Extract of *Adansonia digitata* L. Stem Bark in *Plasmodium berghei*-infected Mice

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Abstract

Increase in the resistance of malaria parasite to synthetic drugs has led to the increasing search for alternative treatment strategy from plant sources. This study investigated the suppressive and prophylactic potentials of flavonoid-rich extracts of *Adansonia digitata* stem bark in *Plasmodium berghei* infected mice. The albino mice were administered with two different doses (200 and 400 mg kg^{-1} b.wt.) of extract for five consecutive days. About 5 mg kg^{-1} b.wt. day^{-1} dose of artemether-lumefantrine and 5 mg kg^{-1} b.wt. day^{-1} dose of chloroquine were used as reference drugs while the control mice received only the vehicle (5% v/v tween 80). In the prophylactic groups, the mice were pretreated daily for five days before they were challenged with inoculums of 1×10^7 chloroquine-sensitive *P. berghei* infected erythrocyte intraperitoneally. The results showed a dose dependent chemosupression in the extract treated groups. The 400 mg kg^{-1} b.wt., was more effective with respect to the parasite clearance than the 200 mg kg^{-1} b.wt., dose. The chemosupression caused by Artemether-Lumefantrine (AL) and chloroquine (CQ) treated groups were significantly ($p<0.05$) higher than the extract-treated groups. The percentage parasitemia also decreased in this manner. The flavonoid-rich extract of *Adansonia digitata* caused a mutual delay in parasitemia. The Packed Cell Volume (PCV) increased significantly ($p<0.05$) in the AL and CQ and 400 mg kg^{-1} b.wt., dose of the extract, respectively when compared with the control. This study showed that flavonoid-rich extract of *Adansonia digitata* stem bark has potent antimalarial property which could be of future importance in malaria management.

Key words: Chemosupression, parasitemia, antimalaria, *Plasmodium berghei*, *Adansonia digitata*, chloroquine, artemether-lumefantrine, packed cell volume

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Malaria continues to exact a devastating toll on the health of human populations worldwide, mostly among children under the age of five. The prevalence of malaria in the tropical and subtropical regions have been attributed to rainfall, consistent high temperatures and high humidity as well as the presence of stagnant waters in which mosquito larvae readily mature, thus providing a favourable environment for the continuous breeding of this vector (Jamieson *et al*., 2006).

According to the World Health Organization (WHO) an estimated 3.3 billion people are at risk of being infected with malaria globally. Epidemiological data estimated that, 198 million cases of malaria occurred globally in 2013 and the disease led to 584000 deaths. The burden is heaviest in the WHO African region, where an estimated 90% of all malaria deaths occur (WHO., 2014).

Artemisinin-based Combination Therapy (ACT) has been adopted as first-line treatment for uncomplicated malaria throughout Africa. The ACTs have been shown to be highly efficacious against asexual parasites and several have been shown to be moderately effective against the gametocyte stage of infection and reduce transmission to mosquitoes. Artemether-Lumefantrine (AL) is the most widely used ACT in Africa. The Dihydroartemisinin-Piperaquine (DP) may be equally efficacious and has advantages of simpler dosing and a longer prophylactic period (Bassat *et al*., 2009).

Transmission reduction is now a key component of global efforts to control and eliminate malaria (Alonso *et al*., 2011). A wide range of novel transmission-reducing drugs and vaccines are currently under development, which aims to reduce malaria incidence by restricting human to mosquito transmission. The transmission-reducing ability of front-line therapeutics such as ACTs or potential combinations of ACTs with gametocytocidal drugs is also gaining increased attention (WHO., 2012; White, 2013).

The emergence and spread of drug resistant malaria parasites in endemic regions has posed a great threat to usefulness of chloroquine (CQ) and Sulphadoxine-Pyrimethamine (SP) the cheapest and widely used antimalarial drugs.

Presently, most malaria endemic countries in Africa including Nigeria have changed their first line antimalarial treatment from CQ or SP to amodiaquine combined with artesunate or the combination of artemether and lumefantrine. The ACTs used in most malaria endemic countries have demonstrated high efficacy, protection against the development of resistance to each component and reduction of malaria transmission (White, 1998; Bloland *et al*., 2000; Sutherland *et al*., 2005). However, the relatively high of costs, dosing complexity and the limited experience of their use in sub-Saharan Africa may hamper the widespread deployment of these drug combinations (Bloland *et al*., 2003).

The prevalence of malaria as well as the growing incidence of deaths resulting from the disease coupled with the increase in the resistance of malaria parasite to synthetic drugs has led to the increasing search for alternative treatment strategy. Plants constitute a natural reservoir of phytochemicals with potentials for the treatment/management of many diseases. The body protective effect from chronic and oxidative stress related diseases have largely been attributed to flavonoids, which are ubiquitously present in plant-derived foods (medicinal plant) and are important constituent of the human health (Digiovanni, 1990; Hertog *et al*., 1993; Knekt *et al*., 1997). The structure-function relationship of flavonoids has been studied extensively to provide an inspiration for the design of a rational drug and/or chemopreventive agent for future pharmaceuticals (Ferte *et al*., 1999).

Adansonia digitata (Bombacaceae) is the most widespread of the *Adansonia* species found in the hot and dry savannahs of sub-Saharan Africa. It has a rich history of ethnobotanical and ethnopharmacological usage in the treatment of a wide range of illnesses including malaria. The stem bark of *A. digitata* is widely used in traditional medicine as a substitute for quinine in case of fever or as a prophylactic (De Caluwe *et al*., 2010).

Various part of the plant is reported to have numerous medicinal and nutritional uses (Von Maydell, 1990). The leaves, bark and fruit pulp have been traditionally used as immunostimulants, analgesics etc., in the treatment of diseases like fever, diarrhoea, cough, dysentery, tuberculosis, microbial infection and worms (Vermaak *et al*., 2011).

The seeds and oil are used as food, fuel, cosmetics and medicines in the tropical treatment of muscle wounds, dandruff and other skin ailments (Chivandi *et al*., 2008; Kamatou *et al*., 2011).

Earlier studies by Ajaiyeoba (2005), Musila *et al*. (2013) and Adeoye and Bewaji (2015) suggested that the stem bark extract of *A. digitata* has significant antimalarial properties. However, few reports exist in the literature on the antimalarial activity of flavonoids-rich extract of *Adansonia digitata* stem bark. Therefore, report the suppressive and prophylactic potentials of flavonoids-rich extract of *Adansonia digitata* stem bark in *Plasmodium berghei* infected mice.

MATERIALS AND METHODS

Plant material: The stem of *Adansonia digitata* (Bombacaceae) was collected from Ido-Ekiti, Ekiti State Nigeria. The plant was identified and authenticated by Mr. Adeniyi K.A. and Mr. Soyewo L.T. in the herbarium unit of Forest Research Institute of Nigeria (FRIN) with identification number No. FHI 109806.

Experimental animals: Twenty five albino mice weighing between 18-20 g were obtained from the animal house, Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were acclimatized for two weeks in the animal house and fed *ad libitum* on commercial rat chow and water throughout the period of the experiment.

Preparation of flavonoid-rich extract: Extraction of flavonoid-rich extract of *Adansonia digitata* stem bark was carried out according to the method described by Chu *et al*. (2002). Exactly 3 g of the crude extract was dissolved in 20 mL of 10% H_2SO_4 in a small flask and was hydrolyzed by heating on a water bath for 30 min at 100°C. The mixture was placed on ice for 15 min, so as to allow the precipitation of the flavonoids aglycones. The cooled solution was filtered and the filterate (flavonoids aglycone mixture) was dissolved in 50 mL of warm 95% ethanol (50°C). The resulting solution was again filtered into 100 mL volumetric flask which was made up to the mark with 95% ethanol. The filtrate collected was concentrated to dryness using a rotary evaporator.

Parasites: The *Plasmodium berghei* was obtained from the Institute of Advanced Medical Research And Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. A standard inoculum of 1×10^7 of parasitized erythrocytes from a donor mouse in volumes of 0.2 mL was used to infect the experimental animals intra-peritoneally.

Transfection and treatment

Early malaria infection/suppressive test: Estimation of the suppressive effects of flavonoid-rich extracts of *Adansonia digitata* stem bark was carried out according to the method described by Peters (1967). Adult albino mice weighing 18–22 g were inoculated by intraperitoneal injection with standard inoculum of *P. berghei* with 1×10^7 infected erythrocytes. The animals were randomly divided into five groups of 5 mice per group and treated for five consecutive days with 200 and 400 mg kg^{-1} b.wt., orally. The Artemether-Lumefantrine (AL) and chloroquine (CQ) groups received 5 mg kg^{-1} b.wt. day^{-1} for five days, while the control received the vehicle (5% v/v tween 80) only. Blood samples were collected from the mice tails and smeared on to microscope slides to make both the thick and thin film. The blood films were first fixed in 100% methanol and then stained with Giemsa prepared with buffered water (pH 7.2). Parasitemia was examined microscopically (using 100x immersion oil objective). The Packed Cell Volume (PCV) was determined on day of infection and day five by the microhematocrit method. Percentage parasitemia and percentage clearance/chemosupression were estimated.

Prophylatic test: Estimation of the prophylatic effects of flavonoids-rich extracts of *Adansonia digitata* stem bark was carried out according to the method described by Peters (1967). The animals were divided into five groups of five mice each and they were pretreated for five days using two different doses (200 and 400 mg kg^{-1} b.wt. day^{-1}). The Artemther-Lumefantrine (AL) and chloroquine (CQ) groups were equally pretreated once with 5 mg kg^{-1} b.wt. day^{-1} for five days, while the control received the vehicle (5% v/v tween 80) only. After five days the mice were transfected intraperitonially with an inoculums size of 1×10^7 of chloroquine sensitive strain of *Plasmodium berghei* infected erythrocytes. After 72 h blood samples were collected from the mice tails and smeared on to microscope slides to make both the thick and thin film. The blood films were first fixed in 100% methanol and then stained with Giemsa prepared with buffered water (pH 7.2). Parasitemia was examined microscopically (using 100x immersion oil objective). Slides were collected for five consecutive days. The Packed Cell Volume (PCV) was determined on day of infection and day five by the microhematocrit method. Percentage parasitemia and percentage clearance/chemosupression were estimated:

$$\text{Percentage parasitemia} = \frac{\text{Total number of parasitized cells}}{\text{Total number of cell}} \times 100$$

$$\frac{\text{Percentage clearance}}{\text{Chemosupression}} = \frac{\text{(Negative control parasitemia)-(Parasitamia with drug)}}{\text{Negative control parasitemia}}$$

Statistical analysis: Results were expressed as mean±standard error of mean. The Duncan multiple range test and student t-test were used to analyze and compared the results at 95% confidence level. Values of $p<0.05$ were considered significant.

RESULTS

Early infection/suppressive test: The percentage parasitemia in the control and the treatment groups were shown in Fig. 1. The flavonoid-rich extract produced a dose-dependent, significant ($p<0.05$) reduction in percentage parasitemia level. The reduction in parasitemia level of 400 mg kg^{-1} b.wt. day^{-1} dose was significantly ($p<0.05$) higher than the 200 mg kg^{-1} b.wt. day^{-1}. A daily increase in parasitemia level in the control group was observed. The parasitemia level in the group treated with artemether-lumefantrine and chloroquine were significantly lower than the extract-treated group.

The flavonoid-rich extract exerted a significant ($p<0.05$), dose-dependent chemosuppressive effect when compared to the control. The extract at 200 mg kg^{-1} b.wt. had 72.39% chemosupression while the 400 mg kg^{-1} b.wt. had 83.63% chemosupression on the fifth day of treatment. The reference drugs, Artemether-Lumefantrine (AL) and chloroquine (CQ) treated group showed the highest chemosupression/clearance and zero parasitemia was achieved on the third day which was maintained throughout the experiment (Fig. 2).

Figure 3 shows the Packed Cell Volume (PCV) in the control and treatment groups. The packed cell volume in the control was significantly ($p<0.05$) lowered (14.00±1.15) when compared with the extract treated groups. The PCV improves in a dose-dependent manner in the extract treated groups.

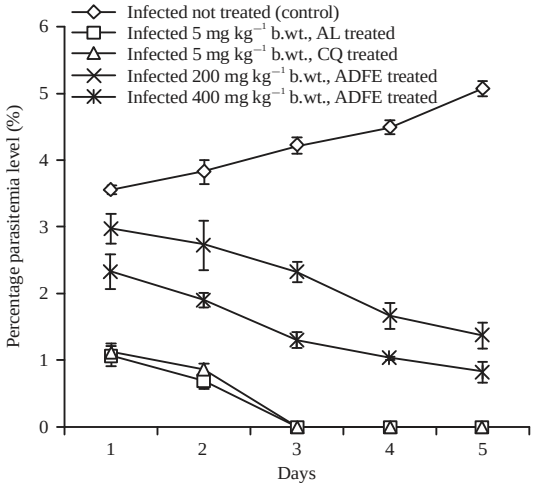

Fig. 1: Percentage parasitemia in *P. berghei* infected mice treated with flavonoid-rich extract of *Adansonia digitata* stem bark, results are expressed as mean of several determinations±standard error of mean (SEM), AL: Artemether-lumefantrine, CQ: Chloroquine

The extract at 400 mg kg^{-1} b.wt., was found to be higher (35.67±0.33) than the 200 mg kg^{-1} b.wt. (31.33±1.76) after the fifth day treatment. The PCV of AL and CQ treated groups improved significantly than the fractions and extract treated groups.

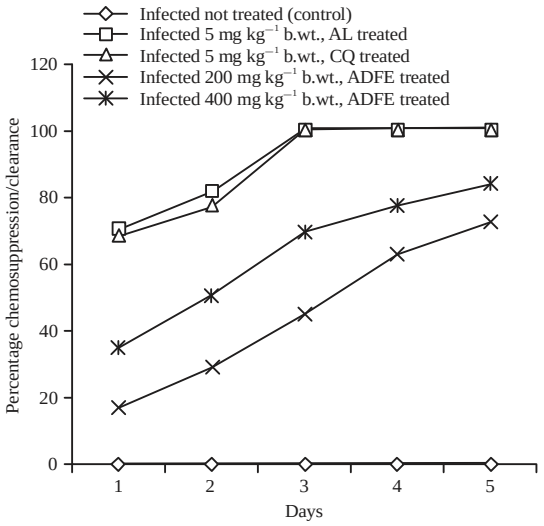

Fig. 2: Percentage clearance/chemosupression in *P. berghei* infected mice treated with flavonoid-rich extract of *Adansonia digitata* stem bark, results are expressed as mean of several determinations±standard error of mean (SEM), AL: Artemether-lumefantrine, CQ: Chloroquine

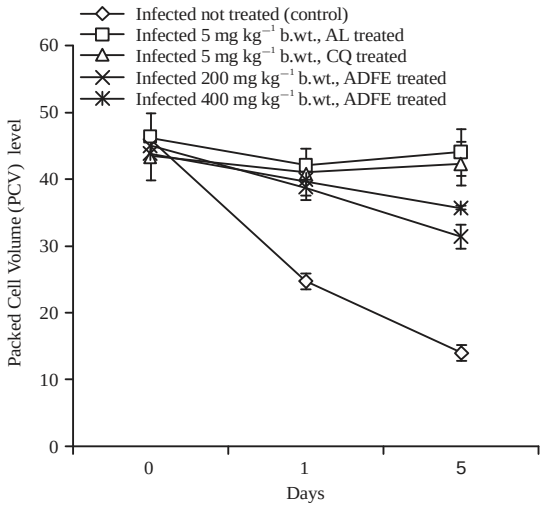

Fig. 3: Packed cell volume of *P. berghei* infected mice treated with flavonoid-rich extract of *Adansonia digitata* stem bark. Results are expressed as mean of several determinations±standard error of mean (SEM), AL: Artemether-lumefantrine, CQ: Chloroquine

Table 1: Prophylactic effects of flavonoid-rich extract of *Adansonia digitata* stem bark on *P. berghei*-infected mice

	1		3		5	
Day	P (%)	C (%)	P (%)	C (%)	P (%)	C (%)
Control	3.75±0.28	-	4.00±0.07	-	5.27±0.19	-
AL 5 mg	0.68±0.07***	81.87±0.00	0.00±0.00***	100.0±0.00	0.00±0.00***	100.0±0.00
CQ 5 mg	1.00±0.12***	73.33±0.00	0.00±0.00***	100.0±0.00	0.00±0.00***	100.0±0.00
ADFE 200 mg	3.00±0.08	20.00±0.00	2.06±0.29***	48.50±0.00	1.67±0.44***	68.31±0.00
ADFE 400 mg	1.90±0.21***	49.33±0.00	1.37±0.09***	65.75±0.00	0.97±0.07***	81.59±0.00

Control (infected untreated) received 5% v/v of tween-80 (the vehicle), 5 mg = 5 mg kg^{-1} b.wt. dose artemether-lumefantrine and chloroquine, 200/400 mg = 200/400 mg kg^{-1} b.wt. dose of *A. digitata* flavonoid-rich extract, %P: Percentage parasitemia, %C: Percentage clearance, AL: Artemether lumefantrine, CQ: Chloroquine, each value is a mean of several determinations±SE after five days of exposure to treatment, ($p<0.05$ was considered statistically significant), ***$p<0.001$ compared with control, untreated group

Table 2: Packed Cell Volume (PCV in percentage) of the prophylactic treated groups

Treatment	Day of infection	Treatment last day
Control	45.80±2.78	15.20±1.77***
AL 5 mg	48.20±2.44	44.20±2.08
CQ 5 mg	46.00±1.79	47.20±1.77
ADFE 200 mg	43.40±2.09	25.00±0.84***
ADFE 400 mg	47.20±2.42	42.80±2.35

Each value is a mean of several determinations±SE after five days of exposure to treatment, $p<0.001$ compared with the respective value on the day of infection, AL: Artemether-lumefantrine, CQ: Chloroquine

Prophylactic test: The prophylactic potential of flavonoid-rich extract of *A. digitata* stem bark on *P. berghei* infected mice is depicted in Table 1. The result revealed that the highest level of the plasmodium parasite was found in the *P. berghei* transfected, untreated mice. The administration of extracts resulted in significant ($p<0.001$) dose-dependent decreases in parasite counts (especially on the 3rd and 5th day) in the treated groups when compared to the control. Significant ($p<0.001$) decreases in the level of the malarial parasites in the blood were only observed in mice pretreated with 400 mg kg^{-1} b.wt., of flavonoid extracts of *A. digitata* stem bark extract after the first day of transfection with the parasites when compared to the control. At this stage, the effect of treatment with 200 mg kg^{-1} b.wt., of the extracts was not significant ($p>0.05$). At day 3 and 5 after transfection however, significant ($p<0.001$) decreases in the level of parasites were afforded by the flavonoid extracts at the two dosages (200 and 400 mg kg^{-1}) evaluated.

The percentage chemosupression for each of the dosage increased with increasing number of days following transfection when compared to that of the control in which there was no suppression.

Furthermore, the effect of the extracts compared well, but lower than those of the reference drugs (5 mg kg^{-1} chloroquine and 5 mg kg^{-1} artemether-lumefantrine) which caused significant ($p<0.001$) decreases right from the first day after transfection (73.33±0.00 and 81.87±0.00, respectively) and 100% clearance at day 3 and 5 after transfection.

The effect of flavonoid-rich extracts of *Adansonia digitata* stem bark on packed cell volume of *P. berghei* infected mice is shown in Table 2. The highest, significant ($p<0.001$) decrease in the PCV level on treatment last day was observed in *berghei* transfected, untreated mice when compared to the level on the first day of treatment. Also, there were significant ($p<0.001$) decreases in PCV levels on the last day of treatment in mice administered 200 mg kg^{-1} b.wt., of flavonoid extracts of *A. digitata* stem bark extract. Treatment with 400 mg kg^{-1} b.wt., of extracts completely reversed the decreases caused by the malaria parasites in the packed cell volume of mice as there were no significant ($p>0.05$) differences in the PCV in both days. Pretreatments with 5 mg kg^{-1} of the reference drugs, chloroquine and artemether/lumefantrine (coartem) reversed the decreases in the PCV of mice caused by the malaria parasites as there were no significant ($p>0.05$) differences when compared to the first day of treatment.

DISCUSSION

The evolution of resistance against available and affordable antimalarial drugs incurs an enormous societal cost for fighting the spread of the disease. However, infections with resistant parasites result in increased morbidity and mortality. So far, no preventative vaccination against malaria exists and its control depends heavily upon antimalarial drugs that kill parasites inside the human body. Treatment strategies of malaria aim to terminate the acute blood infection, cure the clinical symptoms, clear hypnozoites from the liver, prevent future relapses and also prevent the spread of infection.

In the past few years, research in the area of phytotherapy has greatly influenced aspects of nutrition and disease control. The development of new antimalarials from the highly active natural products, which have already been discovered is crucial in order to overcome the increasing resistance of plasmodium to available antimalarials.

The problems of increasing resistance to established antimalarial drugs coupled with the difficulties of the poor

populace to afford and access effective antimalarial drugs have necessitated investigation of chemical compounds from plants for antimalarial properties with the aim of finding new drugs (Ibrahima *et al*., 2012). Recent findings from various studies have boosted the confidence in the once abandoned herbs for the treatment of resistant form of malaria parasites (Avwioro, 2010). Reliance on plants is primarily due to their safety, effectiveness, cultural preferences, inexpensiveness and abundant availability.

The trend of the results showed that all the treatment had effect on the growth of *Plasmodium berghei* parasites in mice. Parasitemia in the control was higher than all the treatment groups. The results demonstrated that the flavonoids-rich extract of *A. digitata* possess blood schizonticidal activity as evident from the chemosupression obtained during the early infection test. This agrees with the work of Ajaiyeoba (2005), Musila *et al*. (2013) and Adeoye and Bewaji (2015).

The observed significant ($p<0.001$) decrease in the level of the parasites in the blood of the mice pretreated with 400 mg kg^{-1} b.wt., of flavonoid-rich extracts of *A. digitata* stem bark after the first day of transfection with the parasite, suggest that *A. digitata* possess significant prophylactic effect against malaria infection in *P. berghei* infected mice. This justifies the ethnomedicinal use of the plant as anti-malarial agent in sub-Saharan Africa (Sidibe and Williams, 2002; De Caluwe *et al*., 2010). In line with the submission of Kamei *et al.* (2000) on the effect of standard antimalarial drugs on mice infected with *P. berghei*, chloroquine (CQ) and Arthemeter/lumefantrine (AL) used in this study exerted 100% suppression on the third day at 5 mg kg^{-1} b.wt. The dose-dependent nature of the antimalarial effect of flavonoids-rich extract of *A. digitata* is evident by the observation that the chemosupression offered increases with increasing concentration of the extracts, with the 400 mg kg^{-1} b.wt., proving to be more effective than the 200 mg kg^{-1} b.wt. This effect may be due to short duration of action of the extract occasioned by rapid metabolism and so parasite clearance could not be total.

The significant reductions in the Packed Cell Volume (PCV) of mice infected with the parasites may be due to combined effect of plasmodial infection and possible destruction or clearance of plasmodial infected blood cells by the administered extracts (Tomas *et al*., 1998). The drop in the PCV that is responsible for malarial anemia occurs both through an increase in the rate at which old red blood cells are broken and a decrease in the rate at which new ones are produced. Plasmodium not only causes the rupture of parasitized red blood cells, but stimulates the activity of macrophages in the spleen, which then destroys both parasitized and unparasitized red blood cells (Umar *et al*., 2013). The malaria-induced reduction in the PCV was completely reversed in mice pretreated with higher dosage (400 mg kg^{-1} b.wt.) of the extract in a comparable manner to the reference drugs supports the anti-malarial efficacy of the intervention, thereby lending credence to the folkloric uses of the plant in this regard.

CONCLUSION

The flavonoid-rich extract of *Adansonia digitata* stem bark has suppressive and prophylactic effect on *Plasmodium berghei* infected mice. It is evident from this study that flavonoid-rich extracts of *A. digitata* stem bark possess potent antimalarial activity which justifies its continuous use in traditional medicine as an antimalarial remedy.

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