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Full Length Research Paper

An investigation of antimalarial potential against Plasmodium berghei in infected mice

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Antimalarial activity of the crude aqueous leaf extract of *Pyrenacantha staudtii* was evaluated using chloroquine-sensitive *Plasmodium berghei* infection in mice with an objective to finding scientific evidence for the use of the plant as traditional antimalarial remedy in Ido/Osi LGA of Ekiti state, Nigeria. The crude aqueous extract of *P. staudtii* Engl. (Icacinaceae) (100, 200 and 500 mg/kg) was administered orally to mice infected with *P. berghei* in 4 days suppressive test. The antiplasmodial effect during the test of the plant in blood was determined and the extract at these doses induced 58.0 to 63.4% activity in comparison with untreated (negative) control group. Chloroquine produced 100% activity. The antimalarial activity showed by *P. staudtii* during the test justifies its use in traditional medicine for treating of malaria in the area.

Key words: *Pyrenacantha staudtii,* herb, malarial, anti-malarial, Ido/Osi, Ekiti, *Plasmodium berghei*, mice.

INTRODUCTION

Malaria continues to be a devastating disease, affecting millions of people living in the endemic areas in the developing world (Hopkins et al., 2007). Numerous attempts have been made to control the disease by using vector control measures and/or chemoprophylaxis, but they have had limited success (Trigg and Kondrachine, 1998). Immunoprophylaxis holds a promise, but effective vaccines are still not available. Presently, the most effective way of dealing with malaria is the administration of chemotherapeutic agents. Control of the main causative agents of malaria; *Plasmodium falciparum* and *Plasmodium vivax,* by use of the classical drugs of chloroquine and primaquine has been frustrated by the resistance of the malarial parasites to these drugs (Foote and Cowman, 1994; Borst and Ouellete, 1995; Garg et al., 1995; Collins and Jefferey, 1996). There is therefore a need to consistently searching for drug with novel modes of action to treat the disease.

Plants have been a great source of medicine useful in the treatment of various diseases (Bako et al., 2005). Therefore, to search for antimalarial drug from plant origin cannot be neglected, more especially, that the antimalarial drugs in use today (quinine and artemisinin) were isolated from plants (Gessler et al., 1994). *Pyrenacantha staudtii* is an annual herb found in the light tropical rain forest and farmland bushes. It is a woody

climber with green influorescent flowers (Falodun and Usifoh, 2006). The plant is widely distributed in south Nigeria and West Cameroons, and across central Africa to Uganda and Angola and it belongs to the family Icacinaceae (Burkill, 1985). The leaves are intensively bitter and the aqueous extract of the plant has been claimed by many traditional medicine practitioners to effectively treat many ailments including malaria, ulcer, gastrointestinal tract infections and threatened abortion (Anosike et al., 2008), dysmenorrheal and intestinal colic (Falodun and Usifoh, 2006).

P. staudtii is traditionally used for the treatment of blemnorrhea, hernia, insomnia, intestinal pain and diarrhea in Nigeria (Awe et al., 2011). The plant was among the plants mentioned to cure malaria among the people of Ido/Osi LGA of Ekiti State, Nigeria (Olorunniyi and Morenikeji, 2013). Since this plant is commonly used in traditional medicine to treat malaria, this experiment was initiated with an objective of investigating its antimalarial potential against *Plasmodium berghei* (NK65) in infected mice.

MATERIALS AND METHODS

Plant collection, authentication and preparation

The fresh leaves of *P. staudtii* were collected from Ajowa Farm at Ido-Ekiti in Ido/Osi LGA of Ekiti State, Nigeria. The plant was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan where voucher specimen was deposited with number FHI No 108805. The leaves of the plants were air-dried inside a room and then grounded into a coarse powder. The coarse powder (200 g) was extracted using distilled water for 48 h at room temperature. The extract was filtered to obtain a filtrate which was concentrated to dryness over a water bath. Appropriate concentrations of the extract were made by serial dilution with distilled water for further experimentation.

Malaria parasites inoculation

Chloroquine sensitive *P. berghei* (Nk65) was obtained from Malaria Drug Research Laboratory, Institute for Advance Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria. Parasites were maintained through serial passage in mice.

In vivo antimalarial test in early infection (4-day suppressive test)

Evaluation of suppressive potential of the extract was done using Knight and Peters 4-day suppressive test against *P. berghei beighei* infection in mice (Knight and Peters, 1980; David et al., 2004). Adult Swiss albino mice weighing 22 to 25 g were injected with 0.2 ml of aliquot 10^6 parasitized erythrocytes, *P. berghei beighei* NK65 intraperitoneally (i.p.). Food and water were provided *ad libitum*. The mice were divided into groups of five per cage. On day 0 (that is, the day of infection), the crude aqueous leaf extract of the plant with the following concentrations (100, 200, 500 mg/kg/day) body weight were administered through oral route 3 h post-infection to every mouse in group 1 to 3, respectively. An initial toxicity test was conducted using the plant extract in which concentration at 500 mg/kg body weight was observed to be saved for the animals. Two control groups were set up which were groups 4 and 5. Mice in group 4 were treated with 10 mg/kg/day chloroquine body weight (Akuodor et al., 2010; Olorunniyi, 2013) to serve as positive control and mice in group 5 were kept untreated but only given water as placebo to serve as negative control. On day 1, 2 and 3, all the animals were treated accordingly (with the same dose and same route) as on day 0. Thin blood smears were prepared on day 11 post-infection. Blood films were fixed in absolute methanol, stained with Giesma stain for 25 mins at pH 7 and then microscopically examined (1000× magnification). Parasitaemia was determined microscopically by counting at least a total number of 1000 uninfected and infected erythrocytes from different fields. Percentage parasitemia was calculated as follows:

$$\text{Percentage parasiteamia} = \frac{\text{No of infected erythrocytes}}{\text{Total No of erythrocytes}} \times 100\%$$

The percentage suppression of parasitaemia was expressed as mean chemosuppression and this was calculated for each dose level by comparing the mean parasitaemia in infected untreated (negative) control with those of treated mice. The difference between the mean value of the control group (taken as 100%) and those of the experimental groups were calculated and expressed as percent reduction or activity using the following equation:

$$\text{Activity} = 100 - \frac{\text{Mean parasitaemia treated}}{\text{Mean parasitaemia (-ve) control}} \times 100\ \%$$

RESULTS

The suppressive activity of the crude aqueous leaf extract of *P. staudtii* against *P. berghei berghei* NK65 in infected mice was examined in early infection (4-day suppressive test). The crude aqueous extract at 100 mg/kg body weight of mice gave 61.6% chemosuppression when compared with the untreated (negative) control group on

Table 1. Antimalarial activity of crude aqueous leaf extract of *Pyrenacantha staudtii* and chloroquine in mice infected with *Plasmodium berghei berghei* NK65 in early infection (4- day suppressive test).

Extract/drug	Dose (mg/kg/day)	Average % parasitaemia	% chemosuppression
	100	2.73 ± 0.67	61.6
P. staudtii	200	2.61 ± 0.5	634
	500	2.99 ± 0.05	58.0
Chloroquine	10	0	100
Control (water)	0.2 L	7.12 ± 1.3	0

Values for parasite density are expressed as mean ± standard deviation (PD ± SD) for five mice per group and the 'activity' when compared with the untreated (negative) control.

day 11 post-infection. At the dose of 200 mg/kg/day, it induced the highest chemosuppression (63.4%) and at 500 mg/kg/day it induced 58%. Chloroquine (reference drug) group had a chemosuppression of 100% (Table 1). Percentage chemosuppression was observed not to be related with increasing the concentrations of the extract. The results showed mean parasitaemia in mice from the ranges of 2.99% ± 0.05 to 2.61% ± 0.5. The mean parasitaemia in chloroquine group was 0 and the mean parasitaemia of untreated control was 7.12% ± 1.3.

DISCUSSION

Crude aqueous leaf extract of *P. staudtii* was observed to show intrinsic antimalarial activity considering its percentage chemosuppression in comparison with the untreated control group in 4-day suppressive test (Knight and Peters, 1980; David et al., 2004). Treatment of mice infected with *P. staudtii* showed no dose-dependent chemosupression in comparison with the untreated control group unlike the results of Ajaiyeoba et al. (2006) in which the activity of methanol extract of *Annona senegalensis* depended on the doses of the extract. The highest chemosupression observed in *P. staudtii* was 200 mg/kg/day treated group of mice. It can be deduced that increasing the concentration of the extract above 100 mg/kg body weight produced no additional suppressive effect against malarial infection. The antimalarial activity showed by *P. staudtii* could be attributable to the presence of alkaloids which was one of its constituents (Anosike et al., 2008). However, the active compound(s) known to give this activity need to be identified. The antimalarial activity showed by *P. staudtii* justifies its use in traditional medicine for treating malaria among the people of Ido/Osi LGA of Ekiti State, Nigeria (Olorunniyi and Morenikeji, 2013), where the plant was collected for the experiment.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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