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Research Paper

IN VIVO ANTIMALARIAL ACTIVITY OF CRUDE AQUEOUS BARK EXTRACT OF *TRICHILIA MONADELPHA* AGAINST *PLASMODIUM BERGHEI BERGHEI* (NK65) IN MICE

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In vivo antimalarial activity of the crude aqueous bark extract of *Trichilia monadelpha* was evaluated using chloroquine-sensitive *Plasmodium berghei berghei* infection in mice with a view 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 *Trichilia monadelpha* (100, 200 and 500 mg/kg) was administered orally to mice infected with *Plasmodium berghei berghei* in both early and established infection. The antiplasmodial effect during early and established infection of the plant in blood was determined. The extract at these doses caused 50-61% activity during early infection and 7.3-24.6% activity during established infection when compared with negative control. Chloroquine produced 100% activity. In both tests percentage parasitaemia still rose but not as high as that of negative control group. *Trichilia monadelpha* seems not to produce a curative effect on malaria but suppresses malaria to a certain degree.

Keywords: *Trichilia monadelpha*, Herbal medicine, Antimalarial, *P. berghei*, Ido/Osi, Mice

INTRODUCTION

Persistence of malaria symptoms after treatment with over the counter available antimalarial drugs has resulted in a gradual loss of faith in orthodox drugs. Within the context of traditional practice, malaria is commonly treated with decoctions or infusions from bitter plants (Milijaona *et al.,* 2009). One possible approach to the identification of new antimalarial drug candidates is to search for compounds that cure or prevent malaria in plants empirically used to treat malaria. Thus, it is worth documenting the ethnobotanical data, and testing the antiplasmodial activity of the extractive from plants (Milijaona *et al.,* 2009). Plants have invariably been a rich source for new drugs and some antimalarial drugs in use today (quinine and artemisinin) were either obtained from plants or developed using their chemical structures as templates (Gessler *et al*., 1994).

Medicinal plant research has become more important, especially after the studies of the

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Chinese antimalarial drug artemisinin, isolated from *Artemisia annua* (O'Neill *et al.,* 1985; Phillipson *et al.,* 1987). More recently a number of studies have been undertaken to evaluate the inhibitory effects of various plant extracts on *P. falciparum* (Presber *et al.*, 1992; Gessler *et al.*, 1994; Moretti *et al.*, 1994; Krugliak *et al.*, 1995) in culture and *P. berghei* in mice (Presber *et al.*, 1992). Various plants used for the treatment of malaria are now reviewed and many of them have been found to be efficacious. Neither the *Cinchona* plants nor *Artemisia annua*, from which the most potent drugs (quinine and artemisinin) were isolated, are indigenous to sub-Saharan Africa, and tropical rainforest plants are known to have higher concentrations of natural chemical defenses and a greater diversity than plants from any other biome, thus they are potential sources of new medicines (Balick *et al*., 1996). It seems logical then to encourage studies on plants from these regions, especially since the major proportion of malaria attributable deaths occur in sub-Saharan African regions. Following this trend, this study was undertaken to evaluate the *in vivo* antiplasmodial activity of *Trichilia monadelpha,* a plant used to treat malaria by the people of Ido/Osi LGA of Ekiti State, Nigeria. *Trichilia monadelpha* (Meliaceae) is an evergreen, small to medium-sized tree up to 20 m tall; bole straight and cylindrical, often low-branching, up to (40-60) cm in diameter, without buttresses; bark surface smooth, pale grey to greenish brown or dark brown, inner bark pale brown to pink; crown spreading, open; young branches short-hairy (Lemmens, 2008). A bark decoction or the pulped bark is applied externally to wounds, sores, skin affections including yaws, lumbago and oedema. A bark decoction is drunk to sooth cough, as an analgesic and anthelmintic, and to treat gonorrhoea and syphilis (Lemmens, 2008). The presence of alkaloids and tannins has been demonstrated for the bark. Limonoids have been isolated from bark and roots (Lemmens, 2008)

MATERIALS AND METHODS

Plant Collection and Authentication

The bark of the plant was 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 108844.

Plant Preparation

The bark of the plant was air-dried inside a ventilated 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 from the plant extract were subsequently made by serial dilution with distilled water for further experimentation.

Malaria Parasites Inoculation

Chloroquine sensitive *Plasmodium beighei beighei* (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.

Evaluation of Schizontocidal Activity in Early Infection (4-day Suppressive Test)

Evaluation of suppressive potential of the extract in early infection was done using Knight and Peters 4-day test against *P. berghei beighei*

infection in mice (Knight and Peters, 1980; David *et al.,* 2004). Adult Swiss albino mice weighing 22-25 g were injected with 0.2 ml of aliquot 10^6 parasitized erythrocytes, *Plasmodium 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 (i.e., the day of infection), the crude aqueous extract of the herbal plant with the following concentrations (500, 200, 100 mg/kg/day) body weight, were administered through oral route 3 h post-infection to every mouse in group 1 to 3, respectively. Two control groups were set up which were groups 4 and 5. Mice in group 4 were treated with chloroquine (10 mg/kg/day) body weight 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.

Evaluation of Schizontocidal Activity in Established Infection (Rane Test)

Evaluation of curative potential of the extracts was done using a method similar to that described by Ryley and Peter (1970). Adult Swiss albino mice weighing 22-25 g were injected with 0.2 ml of aliquot 10^6 parasitized erythrocytes, *Plasmodium beighei berghei* NK65 intraperitoneally (i.p.). Treatment with the crude aqueous extract of the plant only commenced on day 7 post-infection when the infection had been established. The extract of the plant with the following concentrations (500, 200 and 100 mg/kg/day) body weight were administered to mice in groups 1-3, respectively through oral route. The treatment was done repeatedly for four days consecutively. Mice in group 4 were given chloroquine (10 mg/kg/day body wt.) to serve as positive control and mice group 5 were not treated but only given water and to serve as negative control.

Measurement

Thin blood smears were prepared on day 7, 9, 11 ad 15 post-infection of both tests. Blood films were fixed in absolute methanol, stained with Giesma stain for 25 min at pH 7 and then microscopically examined (1000 x 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 (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 activities of the crude aqueous extract of *Trichilia monadelpha* against *P. beighei berghei* NK65 in infected mice were examined in early and established infections. The results are shown in Table 1. The chemosupressive activities of the extract increased with an increase in the concentration in both early and established

infections. It induced the highest activity (61.8%) with 500 mg/kg during the early infection and 24.6% with 500 mg/kg during the established infection on day 15. Chloroquine induced 100% activity in both tests. Figures 1 and 2, respectively show the effect of the crude extract of the plant on malarial suppression during the early and established infection while monitoring the parasitaemia from day 7-15 post-infection.

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

DISCUSSION

Crude aqueous extract of *T. monadelpha* suppresses malaria infection to a certain degree and this justifies its use as antimalarial remedy among the people of Ido/Osi LGA of Ekiti. During the early infection (i.e., 4-day suppressive test), the average percentage parasitaemia of mice administered with 500 mg/kg was 9.5 ± 3.1 which produced the highest activity of 61.8% when compared with the negative water control group that had an average percentage parasitaemia of 24.95 ± 9.2 on day 15 post-infection (Table 1). This method of 4-day suppressive testing for antimalarial activity has become popular (Peters, 1965; Peters *et al.* 1993; Ajaiyeoba *et al.,* 1999; David *et al.,* 2004) during scientific evaluation of potential phyto-medicines for treatment of experimental malaria. In a report of their studies, Ajaiyeoba *et al.* (1999) showed that significant antimalarial activity was deducible from a study of extracts of two plants using the 4 day suppressive *in-vivo* assay method. In this study, there is a decrease in parasitaemia as the concentration of the crude extract of the plant increases from 100-500 mg/kg body weight in the experimental animals. This is similar to the results of Maje *et al*. (2007) on the anti-malarial activity of ethanolic leaf extract of *Paullinia pinnata* during early infections. There was a chemosupression during the established infection but this was not as high as that of early infection.

During the early and established infections, there was a gradual increase in the level of parasitaemia in all the experimental animals and the negative control group while monitoring the percentage parasitaemia from day 7 to 15 post-

Figure 1: Effect of Crude Aqueous Extract of *T. monadelpha* During Early Infection

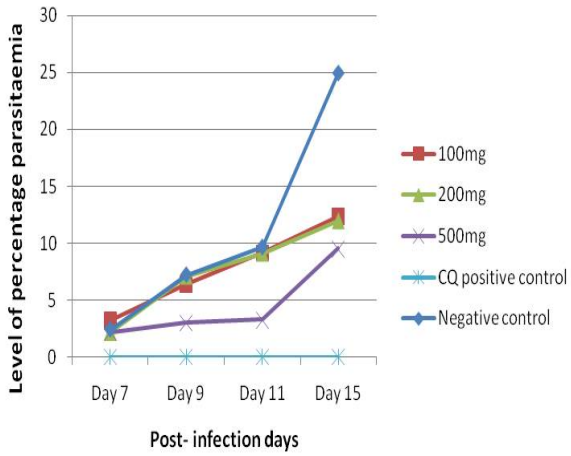

Figure 2: Effect of Crude Aqueous Extract of *T. monadelpha* During Established Infection

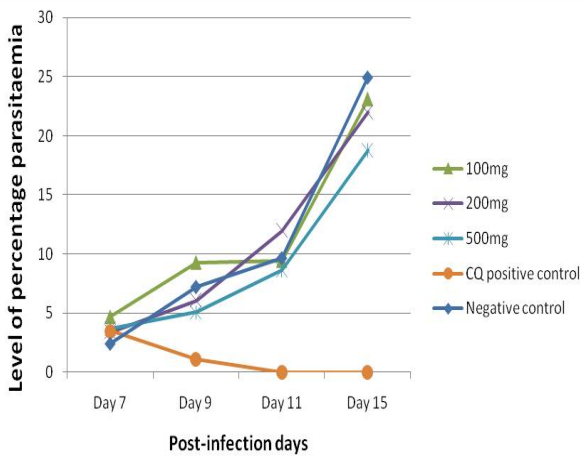

Table 1: Evaluation of Schizontocidal Activity in Early Infection (4-day suppressive test) and Established Infection (Rane test) of *Trichilia monadelpha* (aqueous extract) Treated Albino Mice Infected with *P. berghei berghei* (NK65)

Extract/Drug	Dose (mg/Kg)	Suppressive Test		Rane Test	
		Average % Parasitaemia (day 15p.i.)	Average % Activity	Average % Parasitaemia (day15p.i.)	Average % Activity
T. monadelpha	100	12.3 ± 13.8	50.0	23.13 ± 4.4	7.3
	200	12.0 ± 7.3	52.0	22.0 ± 8.0	11.8
	500	9.5 ± 3.1	61.8	18.8 ± 7.5	24.6
Chloroquine positive control	10	0	100	0	100
Water negative control	0.2 ml	24.95 ± 9.2	0	24.9 ± 9.2	0

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

infection. Whereas, the chloroquine control group showed no parasitaemia on day 15 post-infection in either of the two tests (Figures 1 and 2). For all concentrations of the plant extract in both tests, the curves go up indicating no curative effect against malaria with the plant, unlike the results of Chandel and Bagai (2010) where the extract used, *Ajuga bracteosa* produced a declined curve against percentage parasitaemia to signify anti-malarial properties. Nevertheless, the average percentage parasitaemia levels at the respective concentrations of the extracts were still remarkably lower than the negative control group up till day 15 of post-inoculation. The average parasitaemia was the lowest in 500 mg/kg and highest in negative control (Figure 1). This is an indication that the plant suppresses or hinders parasite growth to a certain degree though this may be small when compared with chloroquine that produced 100% chemosupprresion. The observed higher efficacy of chloroquine may in part be due to non selectivity of the extract or slow absorption and poor bioavailability of the crude extract. Similar observation was reported with the use of medicinal plant extract by Adzu and Haruna (2007) and Iyiola *et al.* (2011). Akuodor *et al.* (2010) obtained more or less similar result during a repository study where treatment of mice infected with *Plasmodium berghei* with ethanolic leaf extract of *Verbena hastata* also exhibited repository activity, but the doses used could not produce a suppression that is comparable to that of the standard drug (chloroquine 10 mg/kg) and a known blood schizonticidal antimalarial agent. However, the level of percentage parasitaemia of negative control group was higher than those of experimental groups. This is a pointer that the plant also has a potency to inhibit the multiplication of malarial parasites, though the effect is small to produce a curative effect. There may be need to identify the active compounds of the plants and if these are concentrated, this could probably produce a curative effect in the treatment of malaria.

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