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Screening *Diospyros mespiliformis* extract for antimalarial potency

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ABSTRACT

Diospyros mespiliformis is used in ethnomedical practice for treating malaria attack. The aqueous extract of the plant's stem bark was investigated for *in vivo* antiplasmodial potency in mice. Curative effect against established infection, suppressive activity against early infection and prophylactic effect in residual infection were tested against *Plasmodium berghei berghei* infected mice. Result shows that the extract (50-200 mg/kg, p.o.) has significant ($p<0.05$) dose dependent activity against the parasite in the curative and suppressive test, and repository effect at high doses (100 and 200 mg/kg, p.o.). The extract also prolonged the survival time of the infected mice. Phytochemical test revealed the presence of saponins, alkaloids, tannins, steroids and terpenes, and the LD_{50} was established to be 1095.4 mg/kg, i.p. in mice. The result shows that the extract has antimalarial potency.

Keywords: *Diospyros mespiliformis*; *Plasmodium berghei berghei*; Phytomedicine

INTRODUCTION

Malaria is caused by the *Plasmodium* genus of protozoa parasites. It is the most important human parasitic infection (Greenwood et al., 2005) with threats to lives in sub-Saharan Africa (WHO, 2005) and little success achieved to control it (White et al., 2004; Lewison and Srivastava, 2008). Traditionally used antimalarials of plant origin have helped in reducing the problems malaria attack posed (Willcox and Bodeker, 2000). Interestingly, some of the plants used have shown real antiparasitic activity (Hilou et al., 2006) and most of them are relatively safe (Ajaiyeoba et al., 2006; Kaou et al., 2008). *Diospyros mespiliformis* Hochst (Ebenaceae) is used in ethnomedical practice against malaria in northern Nigeria (Etkin, 1997). Previous studies in our laboratory showed that the plant has analgesic, anti-inflammatory and antipyretic effects (Adzu et al., 2002a), with CNS activity that is sedative in nature (Adzu et al., 2002b). The plant was also reported to have potent antibacterial (Adeniyi et al., 1990; Lajubutu et al., 1995) and anti-trypanasomal activities (Freiburghaus et al., 1996). In this report, we investigated the ethnomedical usage of the plant as an antimalarial (Etkin, 1997) by evaluating the aqueous extract of the plant's stem bark against *Plasmodium berghei berghei* infected mice. Three experimental models were used: curative effect against established infection; suppressive effect against earlier infection and the repository (prophylactic) potency. The extract's phytochemical constituents and safety level (LD_{50}) were also tested.

MATERIALS AND METHODS

Plant material

Diospyros mespiliformis Hochst (Ebenaceae) ex A.DC--Prodr. (DC.) 8: 672. 1844 [mid Mar 1844] (IK) was collected at Chaza village, Suleja, Niger State, Nigeria in April 2005. It was authenticated by Mal. Ibrahim Muazzam of Taxonomy Unit, Department of Medicinal Plant Research and Traditional Medicine (MPR&TM), National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (NIPRD #5120) was deposited at the herbarium of the Institute. The stem bark of the plant was carefully removed, cleaned and air dried. The dried material was grounded into powder using pestle and mortar, sieved, and 400 g of the material macerated in 1 l of distilled water with occasional stirring using a mechanical shaker (GFL, Germany) for 24 h. The mixture was then filtered, and freeze-dried using Lyovac GT2 (Germany). It gave a yield of 9.74% w/w of crude starting material.

Phytochemical test

The extract was tested for its phytochemical constituents such as tannins, saponins, alkaloids, glycosides, flavanoids, steroids, terpenes and anthocyanines using standard procedures (Evans, 2005).

Animals

Four-week old (Pierrot et al., 2003) Swiss albino mice obtained from Animal Facility Centre (AFC), NIPRD, Abuja, were used for the study. They were housed in standard polypropylene cages with saw dust as beddings under standard conditions, fed on standard pellet feeds, and given water *ad libitum*. The mice were used in accordance with the ethical norms of '*NIH Guide for the Care and Use of Laboratory Animals; NIH Publication (No 83-23), The National Academic Press, Washington, DC (1985).*

Acute toxicity test

The safety of the extract was assessed by testing for its LD_{50} using Lorke's (1983) method. Briefly, the extracts were administered at the doses of 10, 100, 1000 and 2000 mg/kg i.p. to four groups of mice. The mice were then observed for signs and symptoms of toxicity after treatment over a period of 24 h, and deaths within this period were recorded. The LD_{50} was estimated as the square root of the lowest lethal dose multiplied by the highest non-lethal dose from the second stage of dosing (Vongtau et al., 2004).

Rodent parasite *(Plasmodium berghei berghei)* and inoculation

Chloroquine sensitive rodent plasmodia: *Plasmodium berghei berghei* was sourced from National Institute for Medical Research (NIMR), Lagos, Nigeria, by U.A. Katsayal of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University (ABU), Zaria, Nigeria. The parasite was maintained alive in mice at the Department of Parasitology and Entomology, ABU, Zaria by continuous reinfestation (i.p.) (Calvalho et al., 1991) every 4 days. Blood was collected from a donor infected mouse through cardiac puncture and diluted with normal saline. The study mice received 0.2 ml of diluted inoculums i.p. consisting of 10^7 parasitised red blood cell.

Antiplasmodial studies

Curative test

The Rane test (Ryley and Peters, 1970) was used to evaluate the curative potential of the extract against established infection as earlier described (Adzu et al., 2008). Twenty five mice were inoculated as described above, and left untreated until the fourth day (D_4). The mice were grouped into five (n = 5). Groups 1-3 received the extract (50, 100 and 200 mg/kg, p.o.), Group 4 was treated with chloroquine (CQ) (5 mg/kg, p.o.), while group 5 was left untreated and served as the negative control. Treatment then continued daily to D_7 ($D_4 – D_7$) when each mouse was tail-bled, and blood smeared unto a microscope slide to make a thin film. The films were fixed with methanol, stained with 4% Giemsa at pH 7.2 for 45 min and parasitaemia density determined (Kirby et al., 1993). The survival time of the mice in each group over 30 days was noted (Saidu et al., 2000).

Suppressive test

This test on early plasmodial infection (Knight and Peters, 1980) was similar to the curative test described above, except that in this test, treatment started immediately after

the mice were infected with the parasite (Saidu et al., 2000; Okokon and Nwafor, 2009). The mice were then grouped into five (n = 5), and treated with the extract (50, 100 and 200 mg/kg, p.o.), CQ (5 mg/kg, p.o.) and saline (10 ml/kg, p.o.) on that first day (D_1). Treatment continued daily for four days. Thin blood film of each mouse was collected on the fifth day (D_5) and examined for blood parasite suppression, by counting the parasitised red blood cells in random field of the microscope slide of both the treated and control groups.

Repository test

The test was carried out using the residual infection procedure (Peters, 1965). In the test, mice were weighed, grouped into five (n=5) with the mean weight between each group as near as possible, and then treated with the extract (50, 100 and 200 mg/kg, p.o.), CQ (5 mg/kg, p.o.) and saline (10 ml/kg, p.o.), on the first day (D_1). Treatment continued daily for three days (D_1-D_3) and mice were all infected with the parasite on the fourth day (D_4). Thin films of blood smears were made from each mouse 72 h (D_7) after treatment (Abatan and Makinde, 1986) and mean increase/decrease in parasitaemia in each group determined. The mice were reweighed, and changes between pre and post-treatment body weights were noted.

Data analysis

Results were expressed as mean ± SEM. Student's t-test was used to analyse the data between groups and Analysis of Variance (ANOVA) among groups followed by Dunnett's test using GraphPad Prism Version 4.00 for Windows, GraphPad software, San Diego California USA, (www.graphpad.com). $p<0.05$ was considered significant in all cases.

RESULTS

Phytochemical tests

The aqueous extract of *D. mespiliformis* gave positive tests for saponins, tannins, steroids, alkaloids and terpenes.

Acute toxicity tests

The LD_{50} of the extract was established to be 1095. 4 mg/kg, i.p. in mice. Thus, the experimental doses used (50, 100 and 200 mg/kg, p.o.) were within safe margin.

Curative test

There was a dose-dependent reduction in the level of parasitaemia in the treated groups unlike in the saline control group in which there was a consistent increase in the blood parasite density. The mean survival time also increased dose-dependently. Death was observed in the control group on day 7, and by day 10, all mice in the group died (mean survival time of 8 days). On the other hand, mice in the group that received 200 mg/kg survived beyond 21 days. CQ treated group survived the 30 days duration of observation (Table 1).

Suppressive test

The extract exhibited dose dependent suppression of 32.3-73.2% (Table 2).

Repository test

Parasites were detected a day after inoculation in the control group. Mice that received 100 and 200 mg/kg of the extract, and the CQ group showed significant inhibition of the parasite density and gained weight when compared with their pre-treatment data. There was a loss in body weight and inactivity against the parasite in the control and treated groups that received 50 mg/kg of the extract (Table 3).

Table 1: Curative effect of *D. mespiliformis* in *P. bergei* infected mice.

Treatment	Dose (mg/kg, p.o.)	Mean parasitaemia density		Survival time (Days)
		Pre-(D_3)	Post-(D_7)-treatment	
Control	-	33.4 ± 3	41.6 ± 3	8.4 ± 1
Extract	50	28.8 ± 2	13.2 ± 2*	11.2 ± 1
	100	30.4 ± 3	10.4 ± 1*	15.2 ± 1
	200	31.4 ± 4	9.6 ± 1*	22.2 ± 2
CQ	5	29.2 ± 1	2.2 ± 1*	30

D3 = Day three; D7 = Day seven; CQ = chloroquine; *Significantly different: $F[(4,24) = 4.67; p<0.05]$

Table 2: Suppressive activity of *D. mespiliformis* in *P. bergei*-infected mice.

Treatment	Dose (mg/kg, p.o.)	Mean parasitaemia density D_5	% Inhibition
Control	-	32.8 ± 3	-
Extract	50	22.2 ± 2	32.3*
	100	18.4 ± 2	43.9*
	200	8.8 ± 1	73.2*
CQ	5	2.6 ± 1	91.5*

D5 = Day five; CQ = chloroquine;* indicates significantly different: F [(4,24) = 5.0; p < 0.05].

Table 3: Prophylactic activity of *D. Mespiliformis* in *P. bergei*-infected mice.

Treatment	Dose (mg/kg, p.o.)	Mean parasitaemia density D_1	Body Weights (g) D_1	D_7
Control	-	28.4 ± 3	21.94 ± 1	18.8 ± 1*
Extract	50	22.8 ± 4	22.04 ± 1	19.42 ± 2*
	100	16.6 ± 3*	21.92 ± 1	22.12 ± 1
	200	8.2 ± 2*	22.12 ± 2	22.86 ± 1
CQ	5	3.2 ± 1*	22.18 ± 1	24.2 ± 2

D1 = Day one; D7 = Day seven; CQ = chloroquine; *Significantly different: F [(4,24) = 4.45; p < 0.05].

DISCUSSION

The results showed that the aqueous extract of *D. mespiliformis* exhibited significant ($p<0.05$) curative effect against established infection and suppressive potency against early infection of the parasite. It also has prophylactic action at high doses (100 and 200 mg/kg). *P. berghei* has been used in studying the activity of potential antimalarials *in vivo* in rodents (Thomas et al., 1998; Pedroni et al., 2006) and it produces diseases similar to those of human plasmodial infection (English et al., 1996; Kumar et al., 2006). Agents that suppress the parasite (antiplasmodial effect) were known for antimalarial activity (Calvalho et al., 1991; Elufioye and Agbedahunsi, 2004). The mechanism of this antiplasmodial action of the extract at this stage is not clear, however there is that possibility that the agent might have acted through metabolic activation of the immune system (Waako et al., 2005), which can be attributed to the useful phytochemicals in the plant. Plant components like terpenes and alkaloids (detected in the extract), as well as xanthones and flavonoids, were reported to have antiplasmodial effect (Phillipson and Wright, 1990; Christensen and Kharazmi, 2001; Go, 2003). *D. mespiliformis* is used in areas where malaria is endemic, where individuals might possess at least some degree of immunity in which relief may in addition be symptomatic. Our earlier studies on extracts of *D. mespiliformis* showed that it has analgesic, anti-inflammatory, antipyretic and sedative properties (Adzu et al., 2002a, 2002b). Agents with such properties are known to produce additional remedy to malaria patients (Addae-Kyereme et al., 2001). It is plausible that the antiplasmodial activity of the extract might be enhanced by its further purification. Work is presently on going in our laboratories to isolate and characterize the active antiplasmodial component of the plant

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