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Antimalarial potential of the ethanolic leaf extract of *Pseudocedrala kotschyi*

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ABSTRACT

Objective: To establish the efficacy of *Pseudocedrala kotschyi* (*P. kotschyi*) for the treatment of acute malaria attack used in Nigeria. **Methods:** The ethanolic leaf extract was investigated for antimalarial activity against *Plasmodium berghei berghei* (*P. berghei berghei*) in mice. Four-day suppressive, curative effect against established infection and prophylactic models of antiplasmodial studies were used. **Results:** The leaf extract of *P. kotschyi* (100-400 mg/kg b.w. p.o.) exhibited significant dose dependent activity against the parasite in the suppressive and curative, and also had repository activity. The antimalarial effect of *P. kotschyi* is comparable to that of chloroquine. The ethanolic leaf extract also prolonged the survival time of the infected mice. The LD_{50} of the plant extract was established to be $\geqslant$5 000 mg/kg *b.w.* p.o. in mice. **Conclusion:** The results showed that the leaf extract has potential antiplasmodial activity, which can be exploited in malaria therapy.

1. Introduction

Malaria remains the world most devastating human parasitic infection, afflicting more than 500 million people each year[1]. Mortality currently estimated at over a million people per year, has risen in recent years, probably due to increasing resistance to antimlarial medicines[2].

The constant evolution of the malaria parasite has rendered the cheapest and most widely available antimalarial treatments ineffective, especially with the recent reports about the increasing resistance of *Plasmodium falciparum* (*P. falciparum*) to artemisinin-based compounds[3-5].

However, there is deep concern that this parasite will soon develop total resistance to such orthodox treatments. This has led researchers to look for other alternatives, one of which is evaluation of medicinal plants. Therefore, there is an urgent need to explore and utilize the naturally endowed rich biodiversity of communities through research that could translate to benefits for mankind. Hence, such studies on medicinal and beneficial plants could provide useful leads for the synthesis of important active compounds.

Pseudocedrela kotschyi (*P. kotschyi*) is one of such medicinal plants whose therapeutic value no doubt has a folkloric background. The plant is found abundantly in the moisture of heavy soils. It has been used in the treatment of various diseases by traditional healers. Various extracts of the plant have been reported to possess antidiabetic, antiepileptic, analgesic, antipyretic and antimicrobial properties[6-9].

The aim of the current study therefore is to investigate the ethanol leaf extract of *P. kotschyi* for antimalarial potency.

2. Material and methods

2.1. Collection of plant material

Fresh leaves of *P. kotschyi* were collected in the month of

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April, 2010 from chaza village, Suleja, Niger State, Nigeria and duly authenticated in the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria by Dr. (Mrs) Jemilat A. Ibrahim. A specimen of the plant with voucher number (NIPRD/H/6542) was subsequently deposited at the herbarium of NIPRD for reference. The international plant name index is Meliaceae Pseudocedrela kotschyi. Bot. Jahrb. Syst. 22 (1): 154, 1895 (19 Nov. 1895) (IK).

2.2. Extraction of plant material

The leaves were cleaned and taken to the laboratory where they were cut into pieces and air-dried at room temperature for 7 d and ground to powder using mortar and pestle. Three hundred and fifty grams of the grounded leaf powder was then macerated in 1.5 L of ethanol for 24 h and filtered. The filtrate was dried on a water bath at reduced temperature to recover the extract and the yield calculated to be 38 g (11% w/w). The extract was stored in an airtight container and used for the study.

2.3. Phytochemical analysis

The phytochemical screening of ethanol extract of *P. kotschyi* leaf was carried out to determine the presence of the following compounds; alkaloids, tannins, saponins, terpenoides, flavonoids, steroids and cardiac glycosides, carbohydrates, reducing sugar, phlobatannins and anthraquinones using standard procedures[10-12].

2.4. Experimental animals

Swiss albino mice (18-22 g) of both sexes were used for the study. The mice were bred and kept in the Animal House, Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria.

The animals were housed in cages at room temperature and moisture, under naturally illuminated environment of 12:12 h dark/light cycle. They were fed on standard diet and had free access to water. Treatment of the animals was in accordance with the Principles of Laboratory Animal Care (Revised)[13].

2.5. Acute toxicity of the extract

The lethal dose (LD_{50}) of the ethanol leaf extract of *P. kotschyi* was determine in mice using the method as described by Lorke[14] with slight modification. Mice of both sexes were fasted overnight for the toxicity test. The study was done in two phases. In the first phase, 3 groups of 3 mice per cage were administered 10, 100 and 1 000 mg/kg of the extract p.o. The mice were observed for signs of toxicity and mortality for the first 4 h and 24 h. In the second phase, another 3 groups of 3 mice in each cage were further administered 1 600, 2 900 and 5 000 mg/kg of the leaf extract. The mice were also observed for signs of toxicity and mortality at regular intervals for 24 h, 48 h and 72 h respectively.

2.6. Malaria parasites

The chloroquine-sensitive *Plasmodium berghei berghei* (*P. berghei berghei*) (NK65) used to assess the antimalarial activity of *P. kotschyi* leaf was obtained from National Institute for Medical Research (NIMR) Lagos, and kept at the Department of Pharmacology, College of Medical Sciences, University of Calaba, Nigeria. The parasites were maintained by continuous re-infestation in mice.

2.7. Inocula

Parasitized erythrocytes were obtained from a donor infected mouse by cardiac puncture. This was prepared by determining percentage parasitaemia and the erythrocytes count of the donor mouse and diluting them with normal saline in proportions indicated by both determinations[15]. Each mouse was inoculated intraperitoneally with infected blood suspension (0.2 mL) containing 1×10^7 *P. berghei berghei* parasitized red blood cells.

2.8. Suppressive test

A 4--day suppressive test as described by Akuodor et al[16], Mbah et al[17] Was employed for the study. Thirty Swiss albino mice of both sexes weighing (18-22 g) were passaged intraperitonealy with standard inocula of *P. berghei* containing 1×10^7 infected erythrocytes. Four hours after inoculation, the infected mice were randomly divided into 5 groups of 6 mice per cage and treated for four consecutive days (D0-D3). Group 1 received 0.2 mL of normal saline (Drug-free control). Group 2, 3 and 4 received 100, 200 and 400 mg/kg of the ethanol leaf extract respectively, while group 5 received 10 mg/kg of Chloroquine diphosphate. All doses were administered orally. On the fifth day (D4), thin films were made from the tail blood of each mouse.

The films were fixed with methanol, stained with 10%

Giemsa and parasite density determined by microscopically (Olympus CX 21, Japan) counting the parasitized red blood cells on at least 1 000 red blood cells in 10 different fields[15].

2.9. Repository test

This was assessed by using the method described by[18,19]. Thirty Swiss albino mice of both sexes weighing (18-22 g) were randomly divided into 5 groups of 6 mice per cage for ethanol leaf extract of *P. kotschyi*. The mice were orally administered with various doses of the extract (50, 100 and 200 mg/kg), 10 mg/kg chloroquine (reference group) and 0.2 mL normal saline (control group) for three consecutive days.

On the fourth day, the mice were passaged intraperitoneally with standard inocula of *P. berghei berghei* containing $1x10^7$ infected erythrocytes. Seventy-two hours later (D7), thin films were made from the tail blood of each mouse. The films were fixed with methanol, stained with 10% Giemsa and parasitaemia level assessed by microscopically (Olympus CX 21, Japan) counting the parasitized red blood cells on at least 1 000 red blood cells in 10 different fields[15].

2.10. Curative test

On the first day (D0), thirty Swiss albino mice were passaged intraperitonealy with standard inocula of 1×10^7 *P. berghei berghei* infected erythrocytes. Seventy-two hours after, the mice were randomly divided into 5 groups of 6 mice per cage. Group 1 received 0.2 mL of normal saline (Drug-free control). Group 2, 3 and 4 received 100, 200 and 400 mg/kg of the ethanol leaf extract respectively, while group 5 received 10 mg/kg of Chloroquine diphosphate. All doses were administered orally. Treatment continued daily until the seventh day when thin films were made from the tail blood of each mouse.

The films were fixed with methanol, stained with Giemsa and parasitemia density examined by microscopically counting the parasitized red blood cells on at least 1 000 red blood cells in 10 different fields[20,21].

The mean survival time of each group was determined by finding the average survival time (days) of the mice in each group over a period of 28 days (D0-D27).

2.11. Statistical analysis

Results obtained were expressed as mean±S.E.M. The significance of difference between the controls and treated groups were determined using one-way analysis of variance (ANOVA. $P<0.05$ were considered to be statistically significant[22].

3. Results

3.1. Phytochemical test

Results obtained from the phytochemical screening of the ethanol leaf extract of *P. kotschyi* showed the presence of alkaloids, tannins, saponins, terpenoides, flavonoids, steroids, cardiac glycosides, carbohydrates, reducing sugar, while phlobatannins and anthraquinones were absent.

3.2. Acute toxicity test

There was no mortality recorded in the mice upon oral administration even at doses as high as 5 000 mg/kg. This indicates that the experimental doses used are relatively safe.

3.3. Suppressive test

The ethanol leaf extract of *P. kotschyi* demonstrated dose dependent schizonticidal activity at the doses employed in the study. The extract at 400 mg/kg caused 91% suppression as against 90% and 79% suppression induced by 200 and 100 mg/kg respectively. The standard drug chloroquine however caused 94% suppression (Table 1).

Table 1.
Suppressive effect of ethanol leaf extract of *P. kotchyi* against *P. berghei* berghei in mice.

Drug	Dose (mg/kg)	Mean Parasitemia density (D5)	% Suppression
Normal saline	0.2 mL	30.3±0.5	-
P. kotchyi	100	6.4±0.6	79*
	200	3.0±0.3	90*
	400	2.6±0.3	91*
Chloroquine	10	1.7±0.2	94*

D5= Day five, *significantly different from control at $P<0.05$ ($n=6$).

Table 2.
Repository effect of ethanol leaf extract of *P. kotchyi* against *P. berghei* berghei in mice.

Drug	Dose (mg/kg)	Mean Parasitemia density (D7)	% Suppression
Normal saline	0.2 mL	28.9±0.6	-
P. kotchyi	100	7.2±0.3	75*
	200	5.4±0.1	81*
	400	2.9±0.0	90*
Chloroquine	10	1.8±0.2	94*

D7= Day seven, *significantly different from control at $P<0.05$ ($n=6$).

3.4. Repository test

The ethanol leaf extract of *P. kotschyi* exhibited significant

Table 3.
Curative effect of ethanol leaf extract of *P. kotchyi* against *P. berghei* berghei in mice.

Drug	Dose (mg/kg)	Mean parasitemia density		Mean survival time (Days)
		Pre (D3)	Post (D7)	
Normal saline	0.2 mL	30.5±0.6	39.8±0.5	9.3±1.1
P. kotchyi	100	29.0±0.6	10.0±0.3*	22.7±1.9
	200	28.5±0.6	8.7±0.5*	24.3±1.8
	400	30.8±0.5	5.2±0.6*	27.2±1.3
Chloroquine	10	29.8±0.3	3.0±0.3*	28.0±0.0

D3=Day three, D7=Day seven, *significantly different from control at $P<0.05$ ($n=6$). All mice treated with chloroquine survived until day 28.

($P<0.05$) dose dependent reduction in parasitemia density of 75%, 81% and 90% at 100, 200 and 400 mg/kg respectively, whereas chloroquine treated group caused 94% reduction in parasitemia density in the test (Table 2).

3.5. Curative test

There was a dose dependent reduction in the level of parasitemia in the treated group unlike in the saline control group in which there 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 8 and by day 11, all mice in the group died (mean survival time of 9 days). On the other hand, mice in the group that received 100, 200 and 400 mg/kg survived beyond 21 days. Chloroquine treated group survived the 28 days duration of observation (Table 3).

4. Discussion

Phytochemicals constitute an integral part of medicinal plants and are responsible for their numerous bioactivities. Numerous plants containing a wide variety of phytochemicals as their bioactive principle have antiplasmodial activities[23,24]. Although the mechanism of the action of the leaf extract has not been evaluated in the present study, some of the constituents detected have however been implicated in antiplasmodial activities by different mechanisms. The antiplasmodial activity of Berlina grandiflora has been tracked to the alkaloids, flavonoids and terpenoids contained in the plant[20].

The ethanol leaf extract of *P. kotschyi* has been shown to possess schzonticidal activity in vivo following oral administration to infected mice. The extract demonstrated good schozonticidal activity against early infections at the various doses employed in the study.

In the established infection, the ethanol leaf extract at various doses showed significant dose dependant schizonticidal activity. The observed antimalarial activity of the leaf extract is consistent with the traditional use of the plant as herbal medication against the disease and indicative of its potential as a chemotherapeutic antimalarial agent. The plant extract has a noteworthy antimalarial activity as the mean survival time values which at doses used were twice or more than that of control group.

In this study, chloroquine was used as the standard antimalarial drug. Chloroquine has been used for curative, suppressive and prophylactic antiplasmodial activities. In early and established infection, chloroquine interrupts with the heme polymerization by forming a FP-chloroquine complex. This complex is responsible for the disruption of the parasite's cell membrane function and ultimately leads to auto digestion. Though, chloroquine exhibited higher suppressive, prophylactic and curative antiplasmodial activities by the extent of inhibition of parasitemia, the leaf extract of *P. kotschyi* also showed similar antiplasmodial activities.

P. berghei berghei has been used in studying the activity of potential antiplasmodials in vivo in rodents[25,26], and it produces diseases similar to those of human plasmodial infection[27,28]. Agents with suppressive activity against *P. berghei berghei* were known for antiplasmodial activity[29]. Earlier studies on extracts of *P. kotschyi* showed that it possess analgesic and antipyretic properties[7,8]. Agents with such properties are known to produce additional remedy to malaria patients[30].

In conclusion, the extract has demonstrated significant antimalarial activity as seen in its ability to suppress *P. berghei berghei* infection in the three models evaluated. The findings lend pharmacological support to the folkloric use of the plant in the treatment of malaria.

Conflict of interest statement

We declare that we have no conflict of interest.

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