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Investigations of various extracts of *Morinda lucida* for antimalarial actions on *Plasmodium berghei berghei* in mice

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Summary

Morinda lucida extracts, the stem bark, the root bark and the leaves were screened for antimalarial activity in a '4-day schizontocidal test' against a chloroquine-sensitive strain of *P. berghei berghei* in mice. Each extract was administered as a single daily dose on days 0, 1, 2 and 3 to mice that had received an intraperitoneal inoculum of 1×10^7 infected erythrocytes. Each extract produced a degree of suppression of parasitaemia. The most promising result was obtained with chromatographic fractions of the stem bark extracts, the highest dose producing 96.4% suppression of parasitaemia.

Résumé

Les extraits de *Morinda lucida*, l'ecorce de la tige, l'ecorce de la racine, et les feuilles, étaient tirés pour l'activité antipaludéenne dans un '4-jours test schizonticidal' contre une souche de la *Plasmodium berghei berghei* sensible à la chloroquine dans les souris. Chaque extrait était administré journellement en dose unique en jours 0, 1, 2, et 3 chez les souris qui ont reçu un inoculum intraperitoneal de 1×10^7 erythrocytes infectés. Chaque extrait avait produit un degré de suppression de parasitémie. Le résultat le plus prometteur était obtenu avec les fractions chromatographiques de l'extrait de l'écorce du tige, la dose la plus grande a donné 96.4% suppression de la parasitémie.

Introduction

Since 'time immemorial' malaria has been one of the most prevalent of human diseases affecting, particularly, populations of the tropical regions and in the past those of the temperate climates (World Health Organization 1981). However, with the vast increase in international travels there has been a dramatic resurgence of malaria in many countries. Coupled with this is the menacing event of the emergence of *Plasmodium falciparum* resistant to chloroquine and some other antimalarials like proguanil and pyrimethamine (WHO 1965). The malaria situation throughout the world therefore causes great concern. A great need arises at the present time to find compounds that are effective against various drug-resistant strains of *P. falciparum*, tissue schizontocides in order to prevent relapses of *P. vivax* infection and broadly causal prophylactics. With these motives in mind various extracts of *Morinda lucinda* plant were screened against the drug sensitive strain of *P. berghei berghei*. This plant is widely distributed in West Africa and mostly in Western part of Nigeria where it is popularly known as Oruwo. The barks of the root and stem and also leaves are used for the treatment of fevers. The leaves especially after crushing in water and seasoned with salt is drunk in times of fever. (Irvine, 1950; Dalziel, 1948; Watt & Breyer-Branddwjjk, 1962).

Materials and methods

Preparation of various extracts of Morinda lucida

(a) Aqueous root bark extract. Some quantity of the root bark of *Morinda lucinda* was extracted in Methanol. The methanol extract was evaporated to dryness and the residue was dissolved in water. This mixture was filtered and the filtrate concentrated, providing the aqueous extract of the root bark.

(b) Stem bark extracts. 5.25 g of *Morinda lucida* stem bark collected in July was extracted in cold light petroleum for several hours and later with boiling methanol. Further details of this extraction are being published elsewhere. The extraction process yielded various fractions and since they were present in small quantities, they were grouped together for antimalaria investigation as follows:

extraction A = fractions 6–19
extraction B = fractions 20–35
extraction C = fractions 36–40
extraction D = fractions aqueous extract.

The aqueous extract was weighed out and dissolved in distilled water while the other insoluble extracts were suspended in a mixture of 0.5 ml of ethanol and 19.5 ml of distilled water.

(c) Leaf Extract. 67 g of fresh leaves of *Morinda lucida* was collected in late August and extracted with boiling light petroleum for 20 h, concentration gave a dirty brown gummy material. This was the crude extract. Evaluation of the blood Schizontocidal Activity of the various extracts of *Morinda lucida* in mice using the 4-day test. This method is based on that described by Porter and Peters (1975). The blood schizontocidal activity was tested against the drug sensitive *P. berghei berghei* in male albino mice. On day 0, mice were infected intraperitoneally with 0.2 ml inoculum of 1×10^{-7} *P. berghei berghei* infected erythrocytes. Each extract was given as a single daily dose on days 0, 1, 2 and 3 infected controls were 'shamdosed' with an equivalent volume of distilled water. Each dose level consisted of five animals. Parallel tests were run with chloroquine (a standard antimalaria drug) as a reference. The drugs were administered orally through a special cannula. Tail blood films were taken from each animal on day 4. They were stained with Giemsa and the percentage parasitaemia was assessed by microscope counts. All readings were expressed as a percentage suppression of parasitaemia in relation to the control as follows:

$$\left(\frac{(\text{AV\% parasitaemia in controls}) - (\text{AV\% parasitaemia test})}{\text{AV\% parasitaemia in control}}\right) \times 100.$$

The result obtained with chloroquine was plotted on log/probit graph from which the activity of the extracts was extrapolated. The dose of chloroquine and *Morinda lucida* extracts administered were as shown in Table 1.

Results

Evaluation of blood schizontocidal activity of the various extracts

In a preliminary test, the schizontocidal action of the various extracts of *Morinda lucida* administered orally was compared with chloroquine in a 4-day test against sensitivity *P. berghei berghei*. The results of this study are summarized on Tables 2, 3 and 4 and in Figs 1 and 2. A log/probit graph was plotted with the chloroquine results. The ED_{50} of chloroquine extrapolated from the graph was 1.5 mg/kg, (see Fig. 1). Most of the extracts showed some

Table 1. (a) Drug dosage administered

Group	Dose (mg/kg)		
	Chloroquine	Aqueous root bark extract of *Morinda lucida*	Crude light petroleum extracts of *Morinda lucida* leaves
1	10	200	600
2	4	100	300
3	2	50	150
4	0.8	25	—
5	0.32	10	—

(b) Chromatographic fractions of the chloroform extract of *Morinda lucida* stem bark

Group	Drug	Dose (mg/kg)
1	Extract A	800
2		400
3		200
4	Extract B	900
5		450
6		225
7	Extract C	1000
8		500
9		250
10	Extract D	2000
11		1000
12		500

schizontocidal activity. For the chromatographic fractions, extract A (fractions 6–19) gave a suppression of 59.9% at a dose level of 800 mg/kg and 58.6% at 200 mg/kg. The suppression observed here however was not dose dependent. Extract B comprising fractions 20–35 of the original extract gave a high percentage suppression. A dose of 900 mg/kg gave 96.4% suppression while 450 mg/kg gave 74.9% suppression of parasitaemia. When extrapolated from the log/probit graph of chloroquine, the chloroquine equivalent of each was 5.0 mg/kg and 2.6 mg/kg of chloroquine respectively. Extract C (fraction 36–40) however proved toxic at a dose of 1 g/kg. A dose of 500 mg/kg of this extract produced 72.1% suppression with a chloroquine equivalent of 2.5 mg/kg. The highest dose of aqueous stem bark (extract D) at a dose of 2 g/kg was not toxic but gave relatively low suppression of parasitaemia. Much lower activity was observed with aqueous root bark extract (Table 2). The highest dose of the leaf extract (600 mg/kg) suppressed parasitaemia by 51.6% and only low activity was observed with smaller doses (Table 3). Fig. 2 is the histogram of the various extracts

Table 2. Schizontocidal activity of aqueous root bark of *Morinda lucida*

Drug	Dose (mg/kg)	Average % parasitaemia	Average % suppression
Morinda lucida	200	20.7 ± 2.1	7.5
	100	22.0 ± 0.9	1.8
	50	17.3 ± 1.5	22.8
	25	21.0 ± 2.4	6.3
	10	22.8 ± 2.6	−1.8
Control	—	22.4 ± 2.2	0

Table 3. Schizontocidal activity of crude light petroleum extract of *Morinda lucida* leaves

Drug *Morinda lucida*	Dose (mg/kg)	Average % parasitaemia	Average % suppression	Probit	Chloroquine equivalent (mg/kg)
	600	11.3 ± 0.9	51.6	5.040	1.5
	350	15.8 ± 1.5	32.2	4.583	1.00
	150	14.2 ± 1.3	39.1	4.723	1.2
Control		23.3 ± 1.7	0	—	—

Table 4. Schizontocidal activities of the aqueous extract and chromatographic fractions of the chloroform extract of *Morinda lucida* stem bark in '4-day-test'

Drug	Dose (mg/kg)	Average % parasitaemia	Average % suppression	Probit	Chloroquine equivalent (mg/kg)
Extract A	800	10.1 ± 1.8	59.8	5.248	1.8
	400	10.2 ± 1.9	59.3	5.235	1.8
	200	10.4 ± 3.2	58.6	5.217	1.8
Extract B	900	0.9 ± 0.7	96.4	6.799	5.0
	450	6.3 ± 3.2	74.9	5.671	2.6
	225	1.5 ± 1.1	94.0	6.555	4.4
Exract C	1000	Toxic	—	—	—
	500	7.0 ± 4.7	72.1	5.586	2.5
	250	11.0 ± 1.3	56.2	5.156	1.7
Aqueous extract	2000	13.3 ± 4.1	47.0	4.925	1.4
	1000	22.0 ± 4.1	12.4	3.845	0.5
	500	17.3 ± 1.5	31.1	4.507	1.0
Control	—	25.1 ± 3.1	0.0	—	—

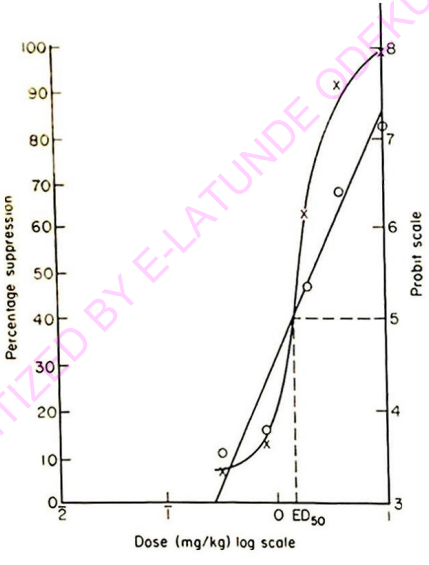

Fig. 1. Determination of ED_{50} of chloroquine. The drug was administered from D0 to D+3 orally and percentage suppression (X) was assessed on DO+4 (O) probit response.

and their schizontocidal activity compared with chloroquine.

Discussion

In this study an intraperitoneal inoculation of 1×10^7 red blood cells parasitised with *P. berghei berghei* was able to cause infection in mice. This confirms that *P. berghei berghei* model is reliable for chemotherapeutic investigations in malaria. This infection was sensitive to chloroquine, this rules out any possibility that drug resistance to standard antimalarials might be contributing to the result obtained.

Observations made on the schizontocidal activity in '4-day test' with the chromatographic fractions of the stem bark appear quite promising. Extract B, comprising fractions 20–35 gave the highest percentage suppression of parasitaemia. With doses of 900 mg/kg and 450 mg/kg, the percentage suppression were 96.4 and 74.9 respectively. When extra polated from chloroquine log/probit activity graph, the chloroquine equivalent were 5.0 mg/kg and 2.63 mg/kg each. Also demonstrable activities were obtained with other fractions such as A and C.

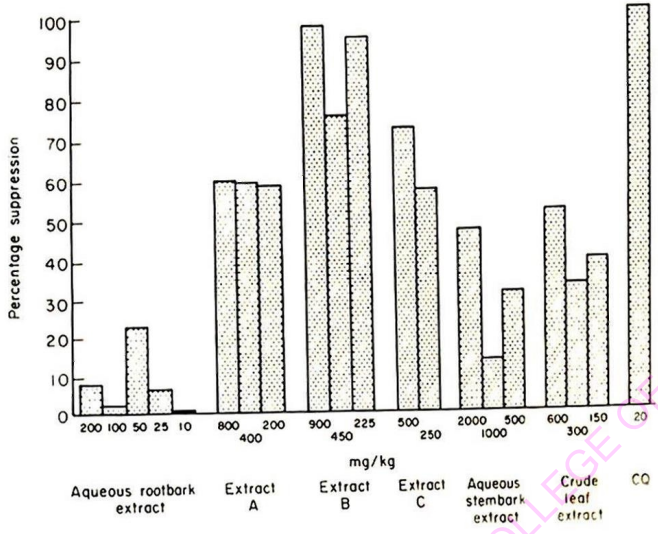

Fig. 2. Effect of subcutaneous administration of chloroquine and *Azadirachta indica* from $D_0 + 3$ to $D_0 + 6$ Percentage parasitaemia in each group was plotted against the days after infection.

The aqueous stem bark extract at a dose of 2000 mg/kg suppressed parasitaemia by only 47%. The highest dose of the aqueous root bark extract gave a parasitaemia suppression of 20.7% 600 mg/kg of crude light petroleum extract of the leaf gave 57.6% suppression. The schizontocidal activity appears to be more concentrated in extract B which was eluted in ether/chloroform. The aqueous component of the stem bark did not show much activity and this may indicate that the schizontocidal activity of the stem bark depends on the extracting solvent.

Conclusion

A promising antimalarial activity has been demonstrated by the various extracts of *Morinda lucida* in a 4-day schizontocidal test. People therefore could not have been in error using this herb for the treatment of fever. In this age where we are experiencing a folorn hope to eradicate malaria totally, coupled with loss of susceptibility by some species of plasmodia to currently used antimalarials, development of new drugs is inevitable. From this study, *Morinda lucida* has proved to be a candidate herb for further antimalarial investigations. The effective extracts of the stem bark and leaves could be further separated in smaller fractions and assessed.

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