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Effect of *Azadirachta indica* on *Plasmodium berghei berghei* in mice

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Summary

Azadirachta indica leaf extract has been investigated for antimalarial activity against drug sensitive strain of *P. berghei berghei* in mice. On administering the extract subcutaneously to infected mice in the '4-day schizontocidal test' 41.2% suppression of parasitaemia was observed. A similar observation was made when the extract was injected for 3 days before the animals were infected with the parasites, 21.7% chemosuppression was obtained. When treatment commenced after the infection had already established, there was no demonstrable suppression of parasitaemia.

Résumé

L'extrait de la feuille d'*Azadirachta indica* était étudié contre une drogue sensible souche du *Plasmodium berghei berghei* pour son activité anti paludisme dans les souris. En administrant l'extrait S.c. chez les souris infectés dans le '4-jours test Schizontocidal' une suppression de 41.2% de la parasitaemie était observé. On avait l'observation semblable quand l'extrait était injecté pour 3 jours avant que les animaux ne sont infectés de parasites, une chémo-suppression de 21.7% était obtenu. Quand le traitement était commencé après l'infection est établi on n'a pas eu suppression demonstrable du parasitaemie.

Introduction

Chemotherapy has been of great importance since the control of malaria was first attempted.

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In the tropical parts of the world and particularly Africa, millions of people are exposed to the administration of various forms of herbal decoctions for the treatment of malaria. True information as to the claims of efficacy of these extracts will contribute in no small measure to knowledge and science while offering at the same time the satisfaction of contributing to the health and well-being of mankind.

Azadirachta indica (Dongo-Yaro-Hausa) is one such plant that is popularly used in the southern part of Nigeria for the treatment of fevers especially presumed malaria. The leaves are boiled and the extract taken in uncontrolled doses. In India it is used as an antipyretic (Symposium, Dakar, 1968). In this study the blood schizontocidal action of this plant against the chloroquine sensitive strain of *P. berghei berghei* in mice was investigated both at the early and established stages of the infection.

Materials and methods

Preparation of Azadirachta indica *extract*

123 g of *Azadirachta indica* leaves were boiled with 500 ml of water for 30 min. The liquid was drained off and reboiled to reduce the final volume to 150 ml.

Evaluation of the blood schizontocidal activity of Azadirachta indica *in mice using the 4-day test*

The technique applied here was similar to that described by Peters (1965) and Porter and Peters (1975). Male albino mice weighing between 18 and 22 g were given a standard inoculum of 1×10^7 erythrocytes infected with *P. berghei berghei* intraperitoneally. In order to

avoid variability in parasitaemia, the animals were infected from one donor mouse. At the same time, these animals were divided into groups of five mice per group. Four groups received subcutaneously the stock of *Azadirachta indica*, one-half stock strength, one-quarter stock strength and one-eighth of the stock strength on days 0 (day of inoculation), 1, 2 and 3. A parallel test was run with chloroquine as a reference and the control animals were given equivalent volume of distilled water. Thin blood films were made from the tail blood of each animal on day 4, stained with Giemsa and examined under the microscope to assess the parasitaemia. The average percentage suppression of parasitaemia in relation to the control was calculated as follows:

$$\text{Average \% suppression} = \frac{\text{Average \% parasitaemia in untreated controls} - \text{Average \% parasitaemia in treated groups}}{\text{Average \% parasitaemia in untreated controls}} \times 100.$$

Evaluation of blood schizontocidal activity in established infection

Since an established infection is less sensitive to treatment than an infection in early stages of development, treatment was withheld in this experiment until parasitaemia was relatively high in order to test whether *Azadirachta indica* is effective at this stage of infection. The method used here was based on that of Ryley and Peters (1970). Male albino Swiss mice weighing between 18 to 20 g received a standard inoculum of 1×10^7 *P. berghei berghei* infected erythrocytes. Seventy-two hours later, the infected animals were divided in groups of five, two groups received once daily subcutaneous injection of *Azadirachta indica* stock and half stock strength for four days. A group received 5 mg/kg of chloroquine as reference and the control animals were sham-dosed with equivalent volume of distilled water. There was daily assessment of parasitaemia from each mouse and time of death of any animal was recorded. The absence of parasites from the blood stream of the animals was taken to be the index of cure.

Repository action

One of the effective methods employed in malaria chemotherapy is chemoprophylaxis. Antimalarial drugs that are long acting have proved very useful. Attempt is made in this work to assess whether *Azadirachta indica* has any repository effect. This method is similar to that described by Peters (1965). Animals were given two dose levels of *Azadirachta indica*, the stock and half the stock strength. Pyrimethamine 1.2 mg/kg, 0.8 mg/kg and 0.3 mg/kg, subcutaneously for 3 days. On the fourth day, these animals were challenged with 1×10^7 erythrocytes parasitized with *P. berghei berghei*. Seventy-two hours after inoculation, tail blood films were made from each animal and percentage suppression of parasitaemia in relation to the control was assessed as described earlier in the 4-day test.

Results

Evaluation of blood schizontocidal activity: 4-day test

The results of this study are summarized on Table 1 and Fig. 1. *Azadirachta indica* extract produced a dose dependent chemosuppressive effect with the highest concentration (stock solution) giving maximum effect of 41.2%. Similarly chloroquine produced a dose dependent chemosuppression with the highest dose (5 mg/kg) giving 100% suppressive effect. The ED_{50} of chloroquine was obtained from the probit line and this was found to be 1.3 mg/kg subject to 95% fiducial range from 2.05 mg/kg to 0.77 mg/kg. The stock solution of *Azadirachta indica* which gave a suppression of 41.2% of parasitaemia, on extrapolating from the

Table 1. Suppressive effect of *Azadirachta indica* on (early infection) 4-day test

Drugs	Average % suppression of parasitaemia
Azadirachta indica	
Stock solution	41.2
½ Stock solution	22.7
¼ Stock solution	16.3
⅛ Stock solution	13.8

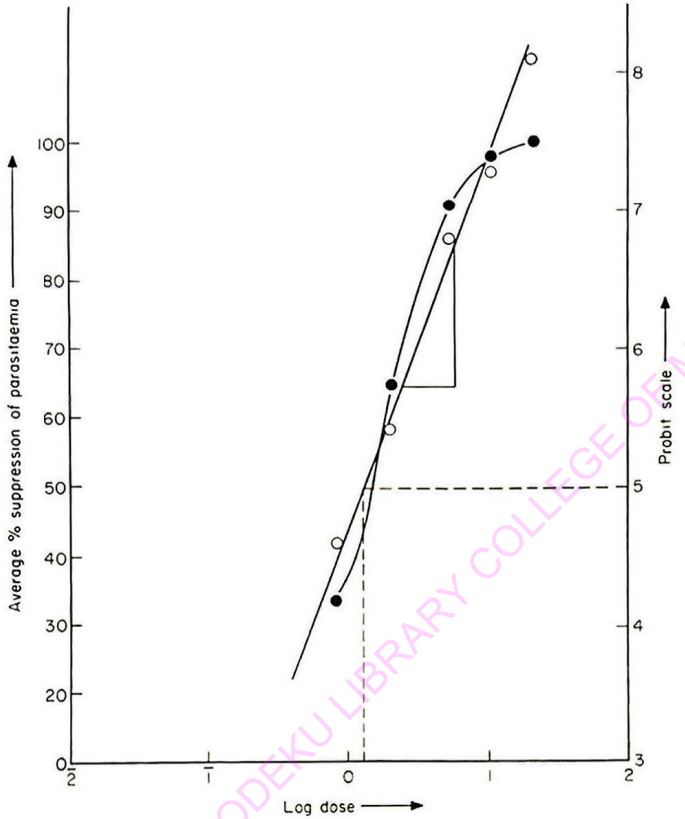

Fig. 1. Percentage suppression of parasitaemia *v.* log dose of chloroquine administered subcutaneously.

chloroquine log/probit line was found to be equivalent to 1.0 mg/kg of chloroquine.

Evaluation of blood schizontocidal activity on established infection

Fig. 2 represents the observation made in this study. The animals that received the stock and half the stock strength, showed a daily rise in parasitaemia before treatment; this was not significantly different from the control. Reduction in parasitaemia was observable after the second day of treatment in animals that received 5 mg/kg of chloroquine. Parasites were completely eliminated in the animals by day 7.

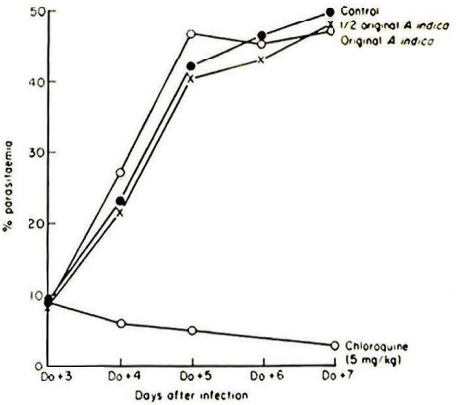

Fig. 2. Percentage parasitaemia in each group *v.* time after infection, Do = days of infection.

Repository action

The results of this work are shown on Table 2. Pyrimethamine at doses of 1.2 and 0.3 mg/kg gave a chemosuppression of 80.5 and 68.1% respectively. The stock solution of *Azadirachta* indica produced 21.7% suppression while half the stock strength produced 5.0% suppression.

Table 2. Repository activity of *Azadirachta indica* and pyrimethamine

Drugs	Average % chemosuppression
Pyrimethamine (mg/kg)	
0.3	68.1
0 8	74 8
1.2	80.5
Azadirachta indica	
Stock solution	21.7
½ Stock solution	5.0

Discussion

The 4-day test offers a good assessment of blood schizontocidal effect of antimalarials. The active antimalarials here are those that are effective on the parasites before they make a strong hold on the host. Both chloroquine and *Azadirachta indica* were found to be effective in suppressing malaria infection in this test. However chloroquine proved to be more active, 5 mg/kg of it abolished parasitaemia completely. Its ED_{50} from the probit line was 1.3 mg/kg. The stock solution of *Azadirachta indica* produced 41.2% chemosuppression and extrapolating this from chloroquine log/probit line was found to be equivalent to 1.0 mg/kg of chloroquine. Evaluation of the residual activity of the aqueous extract of *Azadirachta indica* produced 21.7% against 80.5% suppression observed with 1.2 mg/kg pyrimethamine. The suppressive action of *Azadirachta indica* in early infection agrees with the work of Ekanem (1978) who reported that there was a suppression of parasitaemia using high concentrations of leaf extract. However, in the established infection, the two dose levels of this extract used failed to produce any therapeutic effect. In a parallel test run with chloroquine, a currently used antimalaria, cleared the animals of parasites by the fourth day of treatment using 5 mg/kg subcutaneously. This result indicates that *Azadirachta indica* might not be useful in situations where the infection has already established.

However, Okpanyi and Ezeuku (1981) showed that the bark and leaf extracts of this plant have antipyretic activity. This provides some justification for its use in the treatment of malaria. Therefore therapy with *Azadirachta indica* before parasites establish might lead to early symptomatic relief of fever and thereafter make room for body defence mechanisms to deal with the remaining parasites. This could be contributing to decrease in parasitaemia observed in the early infection (4-day test).

Conclusion

The blood schizontocidal activity of *Azadirachta indica* has been tested both in early and established *P. berghei berghei* malaria. Whereas it was found effective at the early state of infection, it was not effective when malaria had already established. The usefulness of this plant therefore might be dependent on the time of application.

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