

Antiplasmodial effect of three medicinal plants: A preliminary study

Malaria is one of the most prevalent diseases in the world. Each year this disease infects about half a billion people with about 2.3 million deaths¹. WHO experts say that the number of people worldwide infected with malaria is still increasing at the rate of about 5% annually, despite the extensive programmes by the WHO². Mortality and morbidity due to malaria are a matter of great concern throughout the world, especially in tropical and subtropical regions. Even though casualty in children below the age of 5 years is very high, the disease affects all age groups. The pathogenesis occurs during erythrocytic stages. A peculiarity of *Plasmodium falciparum* is its ability to adhere to vascular endothelium (cytoadherence) of erythrocytes infected with maturing parasites. Now the severe and complicated cerebral malaria due to *P. falciparum* is compounded by the chloroquine-resistant parasites. Chloroquine, though effective as a blood schizontocidal, is ineffective or partially effective in resistant cases. Spread of multidrug-resistant strains of *Plasmodium* and the adverse side effects of the existing anti-malarial drugs have necessitated the search for novel, well-tolerated and more efficient anti-malarial drugs^{3,4}. Development of new therapeutic approaches to malaria is very much needed, since resistance of parasites to different anti-malarials is fast developing. The need for an alternative drug initiated intensive efforts for developing new anti-malarials from indigenous plants. Natural products are important sources of biologically active compounds and have potential for development of novel anti-malarial drugs⁵. Natural products are generally safer to mammals, including man. Interest in plant as new anti-malarials has been stimulated by the isolation of artemisinin, a highly active compound against drug-resistant *P. falciparum* from *Artemisia annua*⁶.

The first anti-malarial developed was quinine, prepared from cinchona bark^{7,8}. Synthetic anti-malarials developed later are chloroquine, primaquine, proguanil, pyrimethamine, mefloquine, etc. Para-

sites have developed resistance to almost all of these anti-malarials. This necessitates the need to develop new anti-malarials. There are a series of new synthetic anti-malarials that have been developed and are undergoing different stages of drug trials. Attempts are being made to develop new drugs from medicinal plants. The extract of the bark and leaves of *Azadirachta indica* is being used in Thailand and Nigeria⁹ as anti-malarials since ages. Charaka in 2000 BC and Susruta in 1500 BC reported anti-malarial and antipyretic activity of neem. Ekanem¹⁰ reported schizontocidal activity of aqueous extract of neem leaves on *P. berghei* and Udeinyo¹¹ also found anti-malarial activity in acetone/water extracts of neem leaves on chloroquine-sensitive *P. falciparum*.

Kimbi *et al.*¹² have reported that plants like *Cymbopogon giganteus* (leaves) and *Enantia chlorantha* (bark) which are grown commonly in Nigeria show good potentials against chloroquine-resistant *P. yoelii nigeriensis*, both as schizontocidal and prophylactic agents. The present study reports and confirms the anti-malarial activity of three medicinal plants *A. indica*, *Ocimum sanctum* and *Phyllanthus niruri*.

Three medicinal plants, viz. *A. indica* (stem bark), *P. niruri* (whole plant) and *O. sanctum* (leaves) were used in this study. The plant materials were col-

lected and air dried (in shade) and reduced to powder separately. The extracts were prepared by boiling 100 g of each of the powdered material in 500 ml of double distilled water for 1 h. This decoction was filtered and again boiled and concentrated to 200 ml. The extract was stored at 4°C till use.

Peter's 4-day test^{13,14} was followed to evaluate the blood schizontocidal action against *P. berghei*. In brief, on day-0, experimental as well as control groups of animals were inoculated with 1×10^7 *P. berghei*-infected blood cells and groups of experimental animals were given different doses of test materials consecutively from day-0 to day-3. Blood smears from all the animals were prepared on day-4 and percentage parasitaemia was recorded and compared with that of control animals.

Healthy Swiss Albino Mice (male 4–6 weeks old, approx. 20–25 g) and chloroquine-sensitive *P. berghei* were used in this study. The animals were randomly divided into 6 groups. Each animal was infected intraperitoneally with 1×10^7 *P. berghei*-infected red blood cells in a volume of 0.2 ml diluted in PBS (pH 7.2) on day-0. The first four groups were treated intraperitoneally with the plant extracts in different doses (extract from 100, 300, 500 and 1000 mg dried plant product/kg body weight) as shown in Table 1. The approximate yield, after drying the extracts of 100 mg of the material admin-

Table 1. *In vivo* anti-malarial effect of three medicinal plants

Medicinal plant	Control	Drug dose (in mg)			
		100	300	500	1000
<i>Azadirachta indica</i> (bark; n = 5)	10.32 ± 1.14	8.42 ± 1.02 (18.41)	4.92 ± 0.69 (52.32)	3.64* ± 0.52 (64.72)	3.46* ± 0.8 (66.47)
<i>Phyllanthus niruri</i> (whole plant; n = 3)	10.06 ± 1.95	9.85 ± 0.45 (2.08)	3.7 ± 0.47 (63.22)	3.03 ± 1.47 (69.88)	2.6* ± 1.04 (74.15)
<i>Ocimum sanctum</i> (leaves; n = 3)	6.06 ± 0.84	5.72 ± 0.38 (5.61)	3.96 ± 0.35 (34.65)	3.6 ± 0.28 (40.59)	3.8 ± 0.57 (37.29)

n = number of animals in each group; *Significant at $P < 0.05$; Figures indicate mean percentage parasitaemia; Figures in parentheses indicate percentage reduction in parasitaemia compared to control.

istered from *A. indica*, *P. niruri* and *O. sanctum* was 20, 22 and 25 mg, respectively. The fifth group of mice was given only PBS which served as control. Another control group received chloroquine, 5 mg/kg body weight, as standard anti-malarial. The herbal products/chloroquine/PBS were given consecutively for four days, including day-0. On day-4 thin blood films were prepared from the tail vein of all the animals to monitor the parasitaemia and the reduction of parasitaemia was calculated. Any mortality within 24 h of drug administration was considered as toxicity of the drug.

The suppression of parasitaemia in relation to the control was assessed using the formula by Obih *et al.*¹⁵

Average (Av)% suppression

$$\left[\frac{\text{Av\% parasitaemia in control} - \text{Av\% parasitaemia in test}}{\text{Av\% parasitaemia in control}} \right] \times 100.$$

Percentage parasitaemia at different dosage levels was compared with the control group using Student's *t* test.

The extracts, which were tested for their schizontocidal activity, showed varying results. The parasitaemia on day-4 is shown in Table 1. Even though 100 mg/kg body weight showed negligible effect in all the three groups, 300 and 500 mg/kg body weight showed a reduction of 50–70% parasitaemia. There was not much difference between 500 mg and 1000 mg/kg body weight, except in *P. niruri*. Among the three extracts tested, *P. niruri* showed better results. *P. niruri*, in a dose of 1000 mg/kg body weight, showed a reduction of 74.15% parasitaemia, whereas *A. indica* showed a reduction of 66.47% and *O. sanctum*, a reduction of 37.29%. The animals in the sixth group, which received chloroquine as positive control, were negative. No mortality was observed within 24 h of drug administration, which indicates the herbal products were non-toxic to mice.

The results of the preliminary studies carried out with the above-mentioned plants are encouraging. The scope for developing anti-malarials from indigenous plants depends on screening of a large number of medicinal plants from different geographical regions, especially from the tribal/rural areas, where

usage of these medicinal plants is more common. Once the anti-plasmodial effect of the plant is confirmed, the active ingredients could be isolated by different extraction methods.

A. indica A. Juss. (Meliaceae) is a large evergreen tree, which grows all over India. It has been used in traditional medicine for its medicinal properties. The neem tree has a reputation throughout India and Asia as an anti-malarial plant¹⁶. Methanol extract of *A. indica* stem bark showed an IC₅₀ of 8.5 µg/ml and 40 µg/ml against 3D7 and Dd2 strains of *P. falciparum* when tested *in vitro*¹⁷, while its dichloromethane extract showed an activity with IC₅₀ of 100–499 µg/ml against *P. falciparum* *in vitro*¹⁸. Nimbolide, one of the limnoids from *A. indica*, has an IC₅₀ of 0.95 µg/ml against *P. falciparum* *in vitro*¹⁹. Gedunin isolated from *A. indica* proved to be the most active compound of a series of 21 natural chemical products obtained from Sudanese traditional anti-malarial plants²⁰. The aqueous extract of leaves of *A. indica* showed significant activity at 125–500 mg/kg against *P. berghei*²¹, while in another study it showed 41.2% parasitaemia suppression¹⁵.

O. sanctum Linn. (Lamiaceae) is a sacred herb worshipped by Hindus in India. Its leaves and roots are believed to help cure malaria^{22–25}. Ethanol extract of aerial parts of *O. sanctum* showed MED₅₀ value of > 2000 µg/ml against chloroquine-sensitive *P. falciparum* strains *in vitro*²⁶. Fifty per cent ethanolic extract of its root showed 24.11% inhibition *in vivo* and 11.62% *in vitro* in *P. berghei*, while the same extract of whole plant, excluding root, showed nil and 29.65% inhibition, respectively²⁷.

P. fraternus wbs. syn. *P. niruri* Linn (Euphorbiaceae) occurs as winter weed throughout the hotter parts of India. Young shoots of this plant are hepatoprotective in nature and are used as remedy for jaundice^{28,29}. In folk medicine its root paste is given to children to cure diarrhoea³⁰. *P. niruri* lacks scientific information in literature as an anti-malarial plant. In a recent study, dichloromethane extracts of *P. niruri* whole plant showed 100% and 81.7% inhibition of *P. falciparum* growth *in vitro* at a concentration of 600 and 6 µg/ml respectively, while ethanol extract showed 100 and 64.5% inhibi-

tion respectively³¹. In the present study, *P. niruri* at a dose of 500 mg (= 110 mg dry weight of extract/kg body weight) showed 70% inhibition in parasitaemia in *P. berghei*-infected mice. Further studies are required to establish the anti-plasmodial effect of *P. niruri*, considering the regional and seasonal variations.

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ACKNOWLEDGEMENTS. We are grateful to Dr S. K. Subbarao, Director, Malaria Research Centre, Delhi and Dr S. D. Seth, Consultant, ICMR, New Delhi for their constant support and encouragement to carry out the study. Thanks are due to Dr Aruna Srivastava for statistical assistance and also to Dr Poonam Sharma, Mr A. P. Semwal, Mr

Umesh Jha, Mr Bhopal Singh and Mr Shiv Charan for their help.

Received 23 August 2000; revised accepted 10 November 2000

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Food of the snail, *Pomacea bridgesi*, introduced in India

Ampullariid snails (*Pomacea bridgesi*) are polyphagous as well as voracious in nature¹. Because of their feeding habit, they compete with some of the indigenous species in many countries where they have been introduced^{2–7}. In some cases the indigenous species have failed to survive due to severe competition with these snails⁶. These snails (*P. bridgesi*) have been introduced in India⁸ in recent years in connection with aquarium trade and there exists every possibility of their escape from the aquaria to the open-air water bodies. Hence an attempt was made to gain knowledge on their foods in India, experimentally, under laboratory conditions with a view to apprehend possible impact of *P. bridgesi* on the natural community concerned.

Three glass aquaria, each measuring 30 × 20 × 26 cm were taken for the experiment. Each aquarium was filled with pond water up to 23 cm of its depth. A total of 30 snails, 10 belonging to each of the three groups – juvenile (14–16 mm), sexually mature (30–32 mm) and aged (38–40 mm) *P. bridgesi* individuals, were released into the aquaria separately as regards to the group. The snails were fed with lettuce (*Lactuca sativa*) for a week. Thereafter,

different kinds of food in different states (Table 1) were supplied to these snails, either singly or in different combinations *ad libitum*. In all cases observations were continued for a period of 10 days. To get the required amount of food according to specifications, necessary steps were taken to maintain the stock in the laboratory. The water along with the faecal pellets and the leftover food materials, skeletons, bones and dead snails if any, was removed from the aquarium regularly at an interval of 24 h.

P. bridgesi, irrespective of their groups, exhibited similar type of food selection and feeding (Table 1). In all cases, animal food was preferred to plant food. However, *Tubifex* sp. and *Branchiodrilus* sp. preferred semi-decomposed food over fresh ones of the same kind. *Tubifex* and *Branchiodrilus* were equally preferred by *P. bridgesi*, though the live individuals were consumed prior to dead ones. Dead earthworms were always eaten first in presence of live and/or dead *Tubifex* and *Branchiodrilus*. The semi-decomposed prawn (decapods) and molluscan flesh, irrespective of species, was preferred to oligochaete worms. The flesh of prawns and molluscs was

equally acceptable to *P. bridgesi*. The order of preference for the fishes was: *Setipinna phasa* > *Xenentodon canicila* > *Puntius ticto* > *Colisa fasciatus* > *Amblylopharyngodon mola* > *Labeo rohita* > *Labeo bata*. The fishes, irrespective of species, were consumed first in presence of prawn and molluscan flesh. *Gallus gallus* was preferred to *Capra hircus*. The order of preference for plant foods was: *L. sativa* > *Solanum tuberosa* > *Rumex vascariarius* > *Brassica juncea* > *Raphanus sativus*. *Cobamba* sp. and *Eichhornia crassipes* were equally preferred.

The results indicate that the snails, *P. bridgesi*, have a wide range of food acceptability. These snails would find no problem to establish their colony in India. Consequently, the native macrophytophagous, zoophagous and microphytophagous species would face severe competition for food, if *P. bridgesi* find access to the natural water bodies. This may lead to extinction of some indigenous species as is evident from the disappearance of different species belonging to the genus *Pila* in certain parts of south-east Asia⁵. Besides, in the long run, to meet their need they may start feeding on the paddy plants, as has been noted in some countries where