J. Protozool. Res., *3*, 40- 45 (1993)

Effects of Aqueous Extracts of *Cymbopogon citratus* in Malaria

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Received 3 November 1992 / Accepted 15 March 1993

Key words: *Cymbopogon citratus*, *Plasmodium yoelii*, erythrocyte infection rate, schizontocides, *Plasmodium falciparum*

ABSTRACT

The anti-malarial effect of an aqueous extract of Cymbopogon citratus, a Nigerian traditional medicinal plant, has been investigated in mice infected with Plasmodium yoelii nigeriensis. The plant extract was found effective in suppressing malarial infection. Infected animals, orally given in drinking water 0.106 - 0.473 g ml^{-1} of the extract for 5 consecutive days after the disease had been established, were observed to be cleared of the malarial parasites 4 days after commencement of treatment. The mice, however, lived thereafter, for another 8-10 days before dying because only a low number of non-parasitized red-blood cells were left in the circulation. When on the other hand, the crude salt was injected i.p in doses of 1.0-1.5 g kg^{-1} for 3 consecutive days, it suppressed the infection during and 2 days after the extract treatment, at which time it was observed that the erythrocyte infection rate (EIR) started rising until the animals died at about 8 days post-treatment, that is, they survived 5-6 days longer than the non-treated (control animals). Chemoprophylactic action of the extract injected i.p prior to infection gave protection for about 72 hours. No adverse reactions were recorded when the drug plant was given orally, but certain reactions were noted when administered i.p to which they survived. The drug plant given i.p has an LD_{50} of 2.32 g kg^{-1} and the plant is schizontocidal in action. Chemical analysis of the extract revealed presence of alkaloids, saponins, tannins and simple sugars.

INTRODUCTION

Malaria constitutes the most widespread parasitic disease which causes the most severe health problem in tropical and subtropical areas of the world. Despite the severity of this disease, the currently available drugs have proved inadequate for both chemotherapy and prophylaxis due to the development of Plasmodium falciparum resistant strains. The latter situation, therefore, calls for an urgent search for new and effective drugs especially from natural and synthetic products.

Cymbopogon citratus, (Family Graminaceae), locally known as lemon grass is of Malaysian and Sri-Lankan origin (Purseglove, 1960). The plant is now cultivated throughout the tropics. The medicinal plant is taken by most people infected with P.falciparum in Nigeria, particularly in the rural areas who claimed to be cured with the decoction of C.citratus. This plant is also widely

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used by traditional medicinal practitioners in the treatment of their patients suffering from the disease. Furthermore, no report is found in the literature on investigations of this plant whether it is efficacious in the treatment of P.falciparum infections. It is for this purpose that the present study was set out to look into the efficacy of the drug plant in the therapy of malaria infections in mice inoculated with P.yoelli nigeriensis and to make critical evaluation of its actions and its potential use in humans infected with P.falciparum.

MATERIALS AND METHODS

Collection of C.citratus and preparation of extract

The grass used was collected fresh from a garden at Ikoyi in Lagos State, Nigeria, identified and authenticated by Dr. Olowokudejo of the Department of Botany, University of Lagos. The rhizome of the lemon grass was properly dried in the sun for 7 days to a constant weight. The dried leaves (30 g) were cut into tiny pieces, and simmered in a flask containing 300 ml of distilled water for 1 h. The extract was filtered hot using Whatman filter paper No 1. The filtrate was then evaporated to dryness and the dried extract kept in a refrigerator (4°C) until ready for use. The concentration of the extract was expressed in g ml^{-1}.

Infection of host and parasite counts

The host, source of parasite and the methods used for infection and parasite counts in mice have been described elsewhere (Agbaje and Onabanjo, 1991). Leishman's stain was used in staining the prepared slides (Onabanjo and Adenmosun, 1986) and parasite counts were made daily at mid-day througnout the period of investigation.

Administration and determination of chemotherapeutic effects of the plant extract

The prepared dried extract of C.citratus was re-suspended in distilled water (pH 5.2) and administered through two different routes to two groups of mice. Eight groups of mice consisting of 5 in each group were injected i.p with doses of 1.0-1.5 g kg^{-1} and four other groups were administered orally at will with doses between 80.0-355.0 g kg^{-1}. The extracts were administered 72 h after inoculation when the disease had been established.

Prophylaxis activity test

Four groups of 5 mice each were used. They were administered i.p with 1.0-1.5 g kg^{-1} of the herbal medicine for 3 consecutive days prior to inoculation with P.yoelii (Agbaje and Onabanjo, 1991). The animals were monitored throughout the study.

Acute toxicity bioassay

Toxicity of the extracts of the medicinal plant was determined by the method of Miller and Tainter (1944). Determination of median lethal dose (LD_{50}) was carried out using the i.p route of administration. Doses of 1.25-4.0 g kg^{-1} of the aqueous extract were given to 4 groups of 5 mice each, which were observed for 24 h and thereafter, for up to 7 days.

Phytochemical screening

Chemical tests were performed according to the methods of Odebiyi and Sofowora (1978) to detect certain substances such as alkaloids and others that may be present in the aqueous extract of C.citratus. For alkaloids, Mayers, Dragendorf's and Wagner's reagents were employed while Salkowski test and Benedict's reagent were used for glycosides and reducing sugars. Saponins, anthraquinones, tannins and phlobatannins were investigated by simple chemical tests.

RESULTS

The course of P.yoelii nigeriensis infection

After inoculation of mice with P.yoelii, a pre-patent period of about 72 h was usually observed. All infections were followed by examination of the blood taken from the tail end of the animals at regular intervals throughout the disease duration. Observations were made of the number of parasites in the blood stream and the general clinical condition of the mice. A daily rise in erythrocyte infection rate (EIR) was observed as the disease progressed, until the animal died 7-8 days after infection.

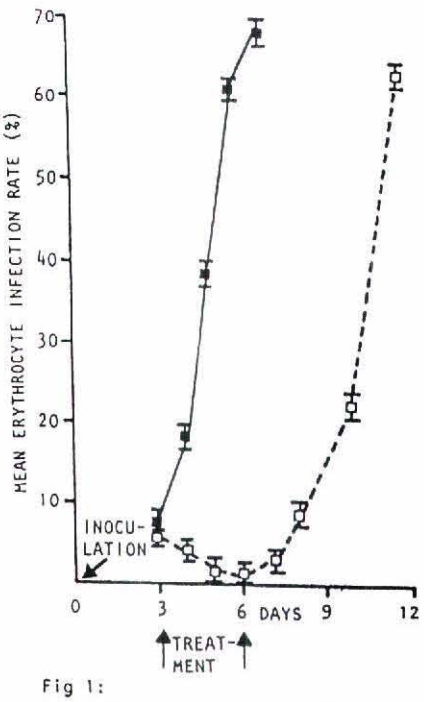

Fig 1:

Chemotherapeutic actions of the aqueous extract of Cymbopogon citratus (□----□) given I.p. to Plasmodium yoelii-infected mice. Control = (■——■). The points on the curves indicate ± SEM. The arrows below the x-axis show the start and end of drug administration.

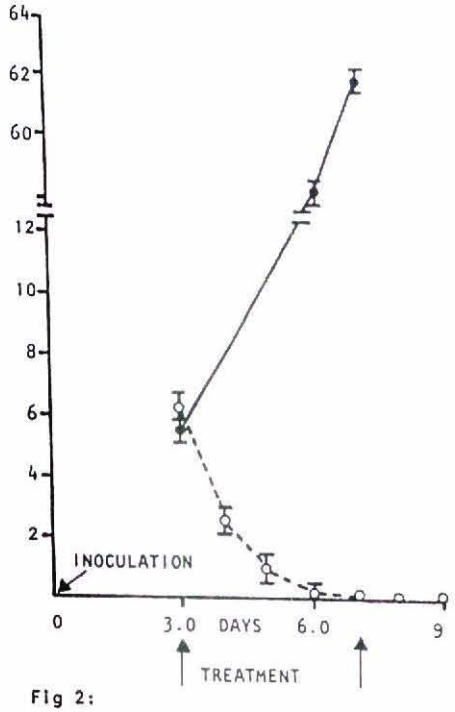

Fig 2:

Antimalarial effects of aqueous extract of C.citratus (O----O) given orally ad-lib to P.yoelii-infected mice. Control = (●——●) The arrows below the x-axis show the start and end of drug administration

Therapeutic effect of C.citratus in P.yoelii-infected mice.

Attempts were made to investigate the doses at which the extract of the drug plant could eliminate malarial parasites from the circulating blood of the mice by injecting i.p (1.0-1.5 g kg^{-1}) the aqueous extract for 3 consecutive days at different parasite levels. Animals with high EIR (>50%) died at about the 5th day post-inoculation, and a similar result was obtained with moderate infection (28-50%). In mice with low EIR (1.0-15%), the parasite level was suppressed and the survival period was prolonged as compared to the controls. However, the i.p route of administration could not produce total cure in the infected animals (Figure 1). When the decoction was given orally ad-libitum for 5 consecutive days, total clearance of the malaria parasites was produced, these animals survived for 10-15 days thereafter, and eventually died (Figure 2).

Prophylaxis study

The animals in this investigation were protected from the disease for 2-3

d longer than the usual pre-patent period. Malaria parasites were later detected in the blood stream of the animals and these survived 7-11 d more than the control (Figure 3).

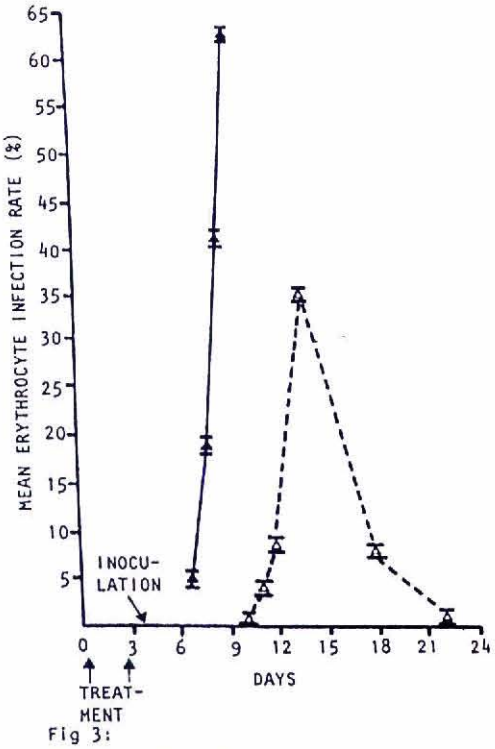

Fig 3:
Prophylactic effect of aqueous extract of C.citratus (Δ---Δ) in P.yoelii-infected mice. Control = (▲——▲). The points on the curves indicate ± S.E.M. The arrows below the x-axis indicate the start and end of drug administration

Toxicity determination

The toxicity of the aqueous extract when administered i.p into mice is shown in Table 1. The LD_{50} value obtained in non-infected animals was 2.32 g kg^{-1} and they exhibited certain reactions which included decreased activity and partial paralysis which lasted for about 12 h. Normal activities resumed in the animals 2 days post-therapy, but some that received higher doses (3.0-4.0 g kg^{-1}) died within 1 h post-extract administration.

Phytochemical assay

Phytochemical analysis of the herbal extract showed that it contains alkaloids, saponins, tannins and sugars.

DISCUSSION

In this study, we found that the aqueous extract of C.citratus was able to induce total suppression of P.yoelii infections in mice which is in agreement with the claims of traditional medicinal practitioners in Nigeria who treat their P.falciparum-infected patients with the leaves of this plant.

In the non-treated acutely infected mice with P.yoelii, a progressive rise in EIR was recorded after a latent period of about 72 h with the animals dying about 4 d later. In the infected mice treated ad-libitum orally with the plant leaf extract, however, the latter was found effective in eliminating the circulating malaria parasites at about the 4th day of treatment (Figure 2).

Table 1. LD_{50} of the aqueous extract of Cymbopogon citratus in mice

Group	Dose (g kg^{-1})	Log dose	Response (%)	Probit
I	1.25	0.097	0	0
II	2.0	0.301	40	4.74
III	3.0	0.477	80	5.84
IV	4.0	0.602	100	7.24

5 mice per group
Route of Administration (i.p)

No toxic reactions were observed with this route in the disease state even when given to non-infected mice, which is in consonance with the experience of traditional healers who claim that the plant did not produce toxic effects when given orally to their patients. Conversely, when non-infected animals were treated i.p with the extract, certain reactions were exhibited which included decreased activity and temporary partial paralysis that lasted for 1-2 h, but they survived. The LD_{50} for i.p administration was 2.32 g kg^{-1} whereas that for oral, given at will, could not be determined since no fatality occurred within 24 h of its administration even at higher concentrations. This may perhaps explain why no toxic reactions have so far been obtained when larger concentrations were given to humans infected with P.falciparum.

The mice infected with P.yoelii and treated orally ad-lib with 80 g kg^{-1} of the extracts were cured of the disease but died 8-10 days after the parasites had been cleared from the circulation. However, in the case of animals given i.p (1.0-1.5 g kg^{-1}) no absolute cure was recorded but the drug plant caused an initial drop followed by a progressive rise in EIR until they succumbed at about the 6th day post-treatment (Figure 1). Fatality obtained in the orally treated mice is thought to be attributed to the low level of the erythrocytes left in the circulation before dying. The effects may be due to lysis of the red cells caused by the extract containing saponins which have been shown to have deleterious haemolysing effect on the circulating erythrocytes (Igweh and Unabanjo, 1989). Death has not been recorded so far in humans infected with P.falciparum and treated with the extract. With i.p administration the extract only produced a short suppression period when injected into the infected animals with low EIR (<15%), whereas no such suppression was recorded when the EIR was greater than 15%. This, however, showed that C.citratus in crude form is not effective when given parenterally at any stage of the infection.

For prophylaxis, the extract given i.p protected the mice for 48-72 h longer than the pre-patent period in the non-treated controls. Nevertheless, parasites were still detected in the blood stream at a high level at about the 10th day post-inoculation and fell markedly to a low level before dying 12 days after the appearance of parasites in the circulation (Figure 3). The latter action might be due to lysis of the red cells. As compared to the controls, the rate of infection in the prophylactic mice was gradual.

Apart from saponins, alkaloids, tannins and reducing sugars were found present in the crude extract. Thus, it seems possible that the alkaloidal content in the extract is responsible for the antimalarial action of the drug plant. If the plant extract is obtained in pure form, it may compare favourably well with the currently known antimalarials used for suppression and prophylaxis. We

have been able to show in this study the chemotherapeutic effectiveness of C.citratus extract in P.yoelii malaria which also indicates the possibility for its use in human infections with P.falciparum.

ACKNOWLEDGEMENT

The authors wish to thank Mrs Rosa Madyambudzi of the Department of Clinical Pharmacology, *University of Zimbabwe Medical School, Harare* for typing the manuscript.

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