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SCREENING OF THE ANTIMALARIAL ACTIVITY OF PLANTS OF THE CUCURBITACEAE FAMILY

CLÁUDIA ZUANY AMORIM; ARTHUR DIAZ MARQUES & RENATO SERGIO BALÃO CORDEIRO/+

Instituto Oswaldo Cruz, Departamento de Fisiologia e Farmacodinâmica, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil

Crude ethanolic extracts (CEEs) from two species of Cucurbitaceae, Cucurbita maxima *and* Momordica charantia *(commonly called "abóbora moranga" and "melão de São Caetano", respectively) were assayed for antimalarial activity by the 4-d suppressive test. The CEE of dry* C. maxima *seeds showed strong antimalarial activity following oral administration (250 and 500 mg/kg), reducing by 50% the levels of parasitemia in* Plasmodium berghei*-infected mice. Treatment of normal animals with 500 mg/kg of the extract three days before intravenous injection of* P. berghei *caused a significant 30% reduction in parasitemic levels. No effect was observed when the animals were treated with the CEE only on the day of inoculation. Oral administration of the CEE of dry* M. charantia *leaves administered orally was ineffective up to 500 mg/kg in lowering the parasitemic levels of malarious mice.*

Key words: antimalarials – drug therapy – natural products – plant extracts – drug screening – *Plasmodium berghei – Cucurbita maxima – Momordica charantia – Cucurbitaceae*

Over the last three decades of malarial control has relied quite successfully and almost exclusively on the use of home DDT spraying and of antimalarial drugs such as chloroquine and quinine (Bruce-Chwatt, 1985). However, as from the mid eighties, an alarming and constant increase in cases of malaria is being observed not only in Brazil (509,000 people in 1987) but worldwide (WHO, 1988). Thus, the need to discover antimalarial drugs with low toxicity and high efficacy has become imperative and increasingly pressing. Although research on natural products has proved to be a rich source for the production of drugs with different activities (Elisabetsky, 1987), only about 10% of the higher plant species had been studied pharmacologically up to 1977 (Farnsworth & Bingel, 1977). Thus, the purpose of the present study was to investigate the potential antimalarial activity of two plants belonging to the Brazilian flora, *Cucurbita maxima* and *Momordica charantia,* popularly called "abóbora moranga" (pumpkin) and "melão de São Caetano" (São Caetano melon), respectively. Both plants belong to the *Cucurbitaceae* family and are considered to have antiparasitic and antimalarial activity in folk medicine.

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+ Corresponding author.

MATERIALS AND METHODS

The antimalarial activity of the extracts was tested on male and female Swiss-44 mice weighing 20 ± 2 g, provided by the animal house of the "Fundação Oswaldo Cruz", using the 4-d suppressive test of Peters (1980). Animals were injected intravenously with 10^7 red blood cells parasitized by *Plasmodium berghei* (Vincke & Lips, 1948) and different groups were treated immediately with the plant extract, antimalarial drugs or vehicle (day zero; d0). Treatments were repeated on d + 1, d + 2 and d + 3. On d + 4, the percentage of parasitized red blood cells was determined for treated and untreated groups using slides containing blood smears obtained from the tail vein and stained with May-Grunwald-Giemsa. To investigate the extent of metabolization of the crude ethanol extract of *C. maxima,* two experiments were performed. In the first, normal mice were treated with the extract for three days before inoculation. In the second experiment, the mice were treated only on the

day of inoculation. In both experiments, the extracts dose used was 500 mg/kg and parasitemia was determined on d + 4 as described above. Crude ethanolic extracts (CEE) of dry *C. maxima* seeds and of dry *M. charantia* leaves were used for the antimalarial activity test. The material was extracted with absolute ethanol at the 1:10 ratio (plant/solvent, W/W). After extraction, the CEEs were lyophilized and solubilized with Tween-80 at the maximum proportion of 2% (V/V) in saline or distilled water (vehicle). The antimalarial drugs pyrimethamine and pyronaridine were used as positive controls (New Drug Group of the Former Department of Malaria, 1980).

The primary screening of the antimalarial activity of the CEEs was performed by oral administration (p.o.). *C. maxima* was provided by Dr Ricardo Lainetti (Faculdade de Farmácia – UFRJ – RJ) and *M. charantia* by Dr Maria de Almeida Silva (Central de Medicamentos do Ministério da Saúde – Brasília, DF). The results are reported as means ± SD. Data were analyzed statistically by the Student t- test (Snedecor, 1963).

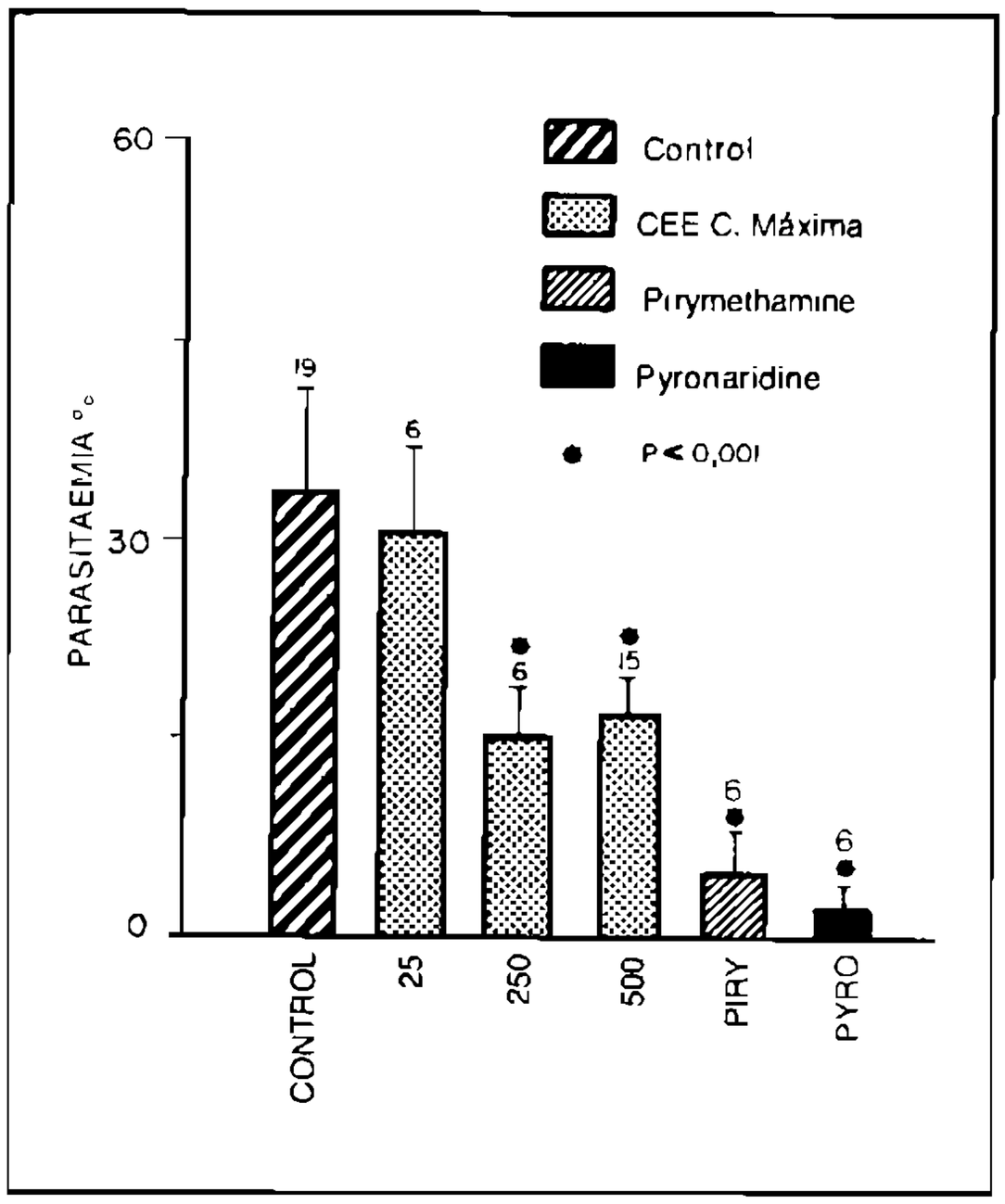

Fig. 1: effect of oral treatment with vehicle (□); ethanolic crude extract (CEE) of *Cucurbita maxima* 25, 50 and 500 mg/kg (□), pyrimethamine 1 mg/kg (□) and pyronaridine 6.8 mg/kg (■) on the parasitemic levels of *Plasmodium berghei*-infected mice. Vertical bars indicate the mean ± SD. Numerals on the bars refer to the number of animals used.

RESULTS

The 4 – day suppressive test (Peters, 1980) for the determination of antimalarial activity showed that the crude ethanol extract of *C. maxima* (Pumpkin) seeds, administered p.o., at the doses of 250 and 500 mg/kg, significantly ($p < 0.001$) reduced by 50% the parasitemia levels of *P. berghei* infected mice, when compared with the saline – treated controls which showed 32% parasitized red blood cells on d + 4. Oral treatment with 1.0 mg/kg pyrimethamine and 6.8 mg/kg pyronaridine (New Drug Group of the Former Department of Malaria, 1980) reduced parasitemia by about 90 and 80%, respectively, (Fig. 1). We also noted that oral treatment of normal animals with 500 mg/kg of the extract or 1 mg/kg pyrimethamine for three days before *Plasmodium* inoculation led to 30 and 90% reduction of parasitemia levels, respectively. However, animals treated with the extract on the day of inoculation (d0) only, no schizonticide activity was observed, (Figs 2, 3). In contrast, the oral administration of CEE of dry *M. charantia* leaves at the doses of 20, 100 and 500 mg/kg did not affect parasitemia levels (Table).

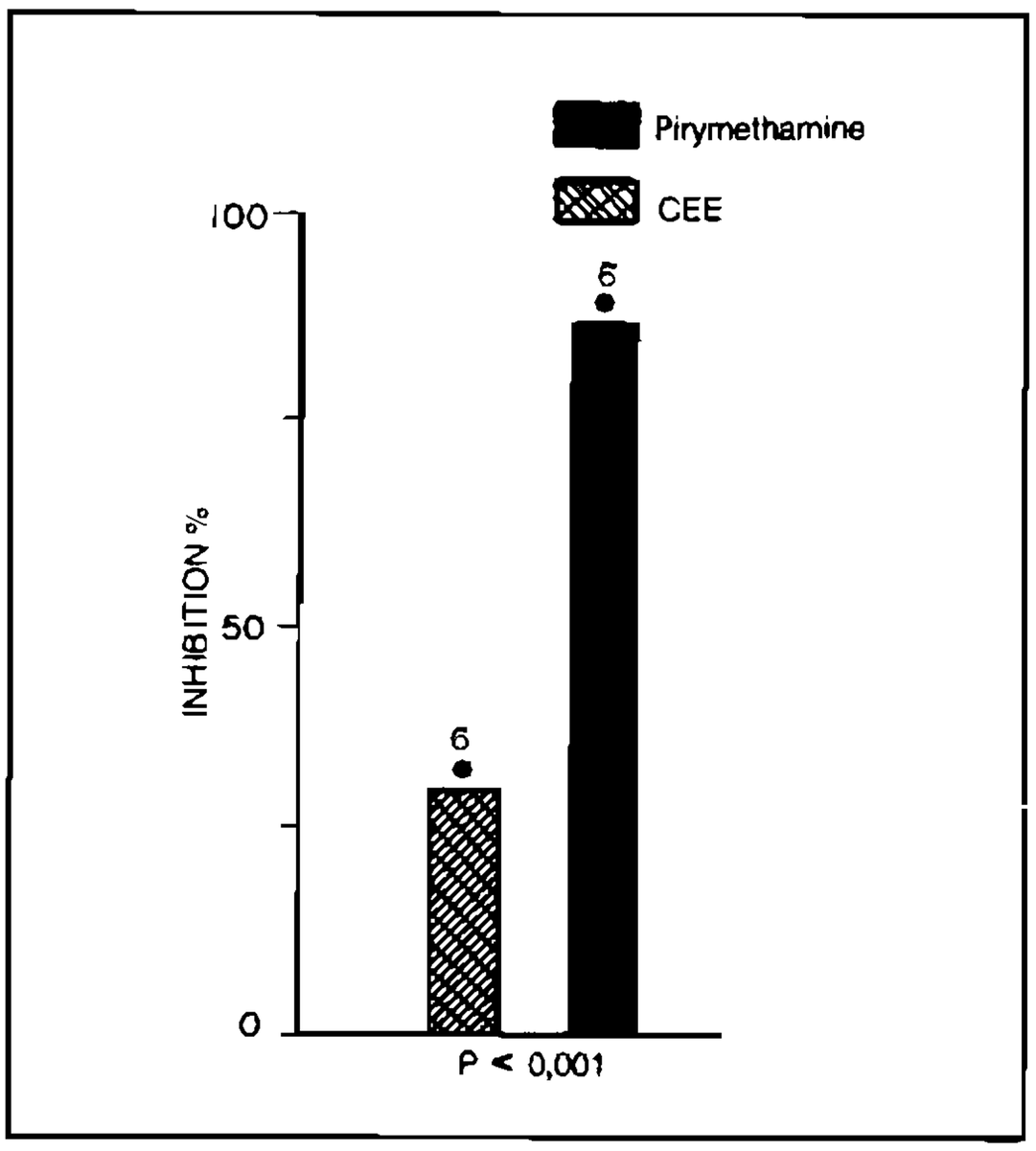

Fig. 2: percent of inhibition of the parasitemic levels of *Plasmodium berghei*-infected mice treated orally with CEE of *Cucurbita maxima* 500 mg/kg (□) and pyrimethamine 1 mg/kg (■) three days before the injection of *Plasmodium*. Vertical bars indicate the mean ± SD. Numerals on the bars refer to the number of animals used.

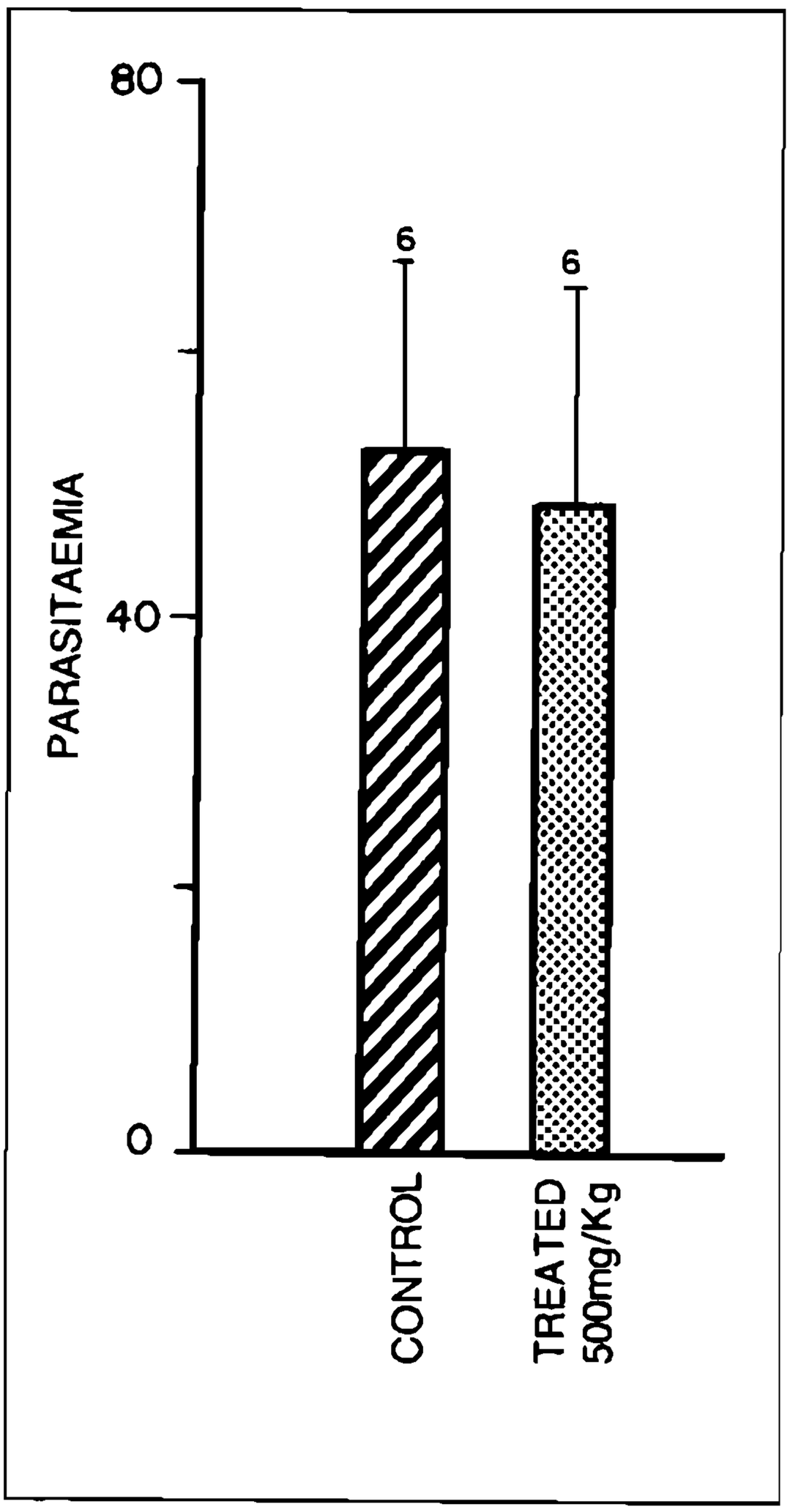

Fig. 3: parasitemic levels of malarious mice treated orally with vehicle (☐) and CEE of *Cucurbita maxima* 500 mg/kg (☐) only on the day of the *Plasmodium* injection. Vertical bars indicate the mean ± SD. Numerals on the bars refer to the number of animals used.

TABLE

Percent of inhibiting effect of ethanolic crude extract of *Mamordica charantia* at the erithrocytic stage of *Plasmodium berghei* on day 4 of malarial infection in mice

Four-day treatment	Route	Dose (mg/kg)	N	Inhibition (%)
M. charantia	p.o.	20	7	0
CEE	p.o.	100	7	0
(leaves)	p.o.	500	7	0

DISCUSSION

Considering the growing prevalence of malarial all over the world and the inability of current efforts to reverse this trend, plants with potential antimalarial activity should be screened in an attempt to detect new drugs with high activity, low toxicity and no resistance to the *Plasmodium*. New promising antimalarial compounds have been discovered in Brazil and in other countries such as *Artemisia annua*, *Simaroubaceae*, *Azadirachta indica*, *Pisum sativum*, *Alstonia boonei*, *Khaya species*, *Morinda lucida*, *Nauclea latifolia*, *Alstonia scholaris* and *Pothomorphe umbellata* (Abatan, 1986; Gbile, 1987; Amorim et al., 1988; Gandhi & Virender, 1990).

In the present study, the CEE from *C. maxima* given orally was effective in preventing the development of parasitaemia in *P. berghei*-infected mice, as evaluated by the 4-d suppressive test. This test is a classical method for the primary screening of drugs with potential antimalarial activity. Furthermore, preliminary observations based on these results have suggested that oral treatment with a *C. maxima* CEE can increase the survival of infected animals (data not shown). Specific experiments for the measurement of survival will be part of a future study. The fact that oral treatment with the CEE of *C. maxima* and with pyrimethamine three days before inoculation with *P. berghei* conferred protection on the infected animals, whereas administration of the extract on the day of inoculation only did not protect them, indicates that these drugs may be stored or undergo slow metabolization. In contrast, the CEE of *M. charantia* a plant belonging to the same family of *C. maxima*, was ineffective in reducing the levels of parasitemia. This finding is in agreement with those reported by Carvalho (1990), who found that aqueous extracts of the plant failed to modify parasitemic levels.

Thus, the investigation of plant derived chemical compounds is of fundamental importance for the development of new antimalarial drugs, especially in view of the vast potential of the Brazilian as well as the worldwide flora and of the outstanding importance of quinine (extracted from the bark of cinchona) in the treatment of malaria.

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