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ORIGINAL PAPER

Effects of homeopathic medications *Eupatorium perfoliatum* and *Arsenicum album* on parasitemia of *Plasmodium berghei*-infected mice

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Malaria is one of the most important parasitic diseases in the world and a major public health problem because of emerging drug-resistant strains of *Plasmodium*. A number of synthetic and natural compounds are now being analysed to develop more effective antimalarial drugs. We investigated the effect of homeopathic preparations of *Eupatorium perfoliatum* and *Arsenicum album* on parasitemia using a rodent malaria model.

We found significant inhibitory effect on parasite multiplication with both medications with a level of 60% for *Eupatorium perfoliatum* at a 30 CH potency. *Arsenicum album* 0/6 gave 70% inhibition but this was less stable than *Eupatorium perfoliatum*. The number of schizonts was higher in animals treated with homeopathic medications. Although the mechanism of action is unknown, these agents would be good candidates as alternative or complementary medications in the treatment of malaria. *Homeopathy* (2006) **95,** 223–228.

Keywords: *Plasmodium berghei; Eupatorium perfoliatum; Arsenicum album;* Balb/c mice; Homeopathic medications

Introduction

Malaria is a major parasitic disease in humans with more than 300 million cases and over 1 million deaths reported every year.[1] Despite numerous attempts to fight malaria, such as control of vectors and chemoprophylaxis, success has been limited. At the present, the most effective way to control this disease is by the use of antimalarial drugs,[2] however, the spread of *Plasmodium falciparum* strains resistant to chloroquine and other anti-malarial drugs, such as mefloquine and sulfadoxine–pyrimethamine combinations, is increasing.[3,4] This makes it imperative that new therapeutic alternatives are sought, especially since some of those strains display multidrug-resistant phenotypes, even against analogues of chloroquine.[4]

We evaluated the effect of homeopathic preparations of *Eupatorium perfoliatum* and *Arsenicum album*, on parasitemia of BALB/c mice infected with *Plasmodium berghei*. This rodent malaria model has been successfully used to evaluate antimalarial properties of several compounds and the results obtained were similar when they were tested against human parasites, such as *Plasmodium falciparum*.[3–7]

Plants belonging to the *Eupatorium* genera have been studied in some depth and several compounds with varying effects identified. Some have analgesic and antiinflamatory properties,[8–11] while others have

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antibacterial, antifungal and virucidal activities.[12 22] Compounds extracted from *Eupatorium inulaefolium* have shown to be effective against *Plasmodium falciparum* in vitro[23] and extracts of *Eupatorium squalidum* have a 40–50% of inhibitory effect on the growth of *Plasmodium berghei* in infected mice.[24]

Homeopathic preparations have been shown to be effective in various situations. For instance the *Eupatorium perfoliatum* extracts show significant cytotoxic effects but weak antibacterial activities against *Staphylococcus aureus* and *Bacillus megaterium*,[25] while homeopathic preparations of *Eupatorium perfoliatum* have been shown to be equally effective in the relief of common cold symptoms as acetylsalicylic acid.[26] *Arsenicum album* (arsenic trioxide As_2O_3) is a toxic salt which reacts with sulphydryl groups of cellular enzymes causing anoxia and death, however in homeopathic preparation it is recommended in the treatment of intermittent fever, specially in malaria when *China officinalis* cannot be used.[27]

Material and methods

Animals

Six to eight weeks old male BALB/c mice from the animal breeding unit of Centro de Investigación y de Estudios Avanzados of Instituto Politécnico Nacional were used . They were maintained on a 12 h light–dark cycle and given free access to food and water. Each mouse was used only once and handling conformed national law NOM-062-ZOO-1999 'Technical Specifications for the Production, Animal Care and Use of Laboratory Animals'. Because after the experiments the mice remained infected with *Plasmodium*, they were euthanized with CO_2 at the end of the protocol following the guidelines of the 2000 American Veterinary Medical Association Panel on Euthanasia.

Parasites

Plasmodium berghei strain ANKA 2.34 was used. The parasites were maintained following the protocol as reported previously[28] and were passaged once or twice in BALB/c mice before use in the experiments.

Homeopathic medications

Eupatorium perfoliatum and *Arsenicum album* from *Similia* laboratories (Similia, México, D.F.) were used. The 30 CH and 0/6 potencies were prepared by Dr Gonzalo Cortés according to Mexican Pharmacopoeia.[29,30] The dose used was 8 µl for each potency diluted in 200 µl of distilled water per dose per mouse.

Treatment protocol

Four groups of four mice each were established. Each mouse was inoculated with 5×10^6 parasites diluted in 200 µl of PBS by the intraperitoneal route. Twenty four hour later the treatment began. The compounds were administrated orally using an orogastric probe (Popper & Sons Inc. New Hyde Park, NY, USA. Catalog number 01-4260-19). One group was treated with vehicle (87° dynamized homeopathic alcohol) as a negative control; another with *Eupatorium perfoliatum* 30 CH, another with *Eupatorium perfoliatum* 0/6 and one with chloroquine (Sigma) (5 mg/kg/day) as a positive control. The medications were administrated every 24 h for 9 days. The same protocol was used for *Arsenicum album*.

Parasitemia evaluation

To evaluate parasitemia, a drop of tail blood was obtained from each mouse and was smeared on two glass slides. The blood cells were fixed with methanol (Omnichem) and stained with 20% Giemsa solution (Sigma) for 5 min. The thin blood smears were observed under light microscopy (magnification × 1000) and the number of infected erythrocytes in 50 fields was counted. To determine the inhibition rate of parasitemia the following formula was used: [(Parasitemia in control group (treated with vehicle)−Parasitemia in drug-treated group)/(Parasitemia in control group) × 100%] according to Su *et al.*[31] Parasitemia was evaluated on days 2, 3, 4, 5 and 9 using thin blood smears stained with Giemsa by microscopy. On day 10 all the mice were sacrificed. No fatalities were recorded during the experiments.

Statistical analysis

Parasitemia is expressed as mean ± SD. The mean and standard deviations of the different treatments were compared between experiment and control groups using one-way ANOVA, while F_c values were compared using a Turkey test ($P<0.05$) with one degree of freedom.[32]

Results

Effect of *Eupatorium perfoliatum*

The mice treated with vehicle alone (control) showed a significant increase in the parasitemia, which started on day 2 and was maximal on day 4. On day 5 it started to decrease (Figure 1A), mainly due to secondary anaemia, as result of the destruction of the red blood cells during the release of the parasites. Since parasitemia is evaluated by the number of infected erythrocytes, secondary anaemia causes an apparent reduction in the total parasitemia.

The effect of chloroquine was immediately obvious. The levels of parasitemia in all members of this group remained lower than the others for all days tested, however, there was a slight increase from day 5 to day 9. This effect has been observed by other authors when a dose of 5 mg/kg/day was used.[33] The mice did not develop secondary anaemia because chloroquine controls parasitemia and more erythrocytes are available

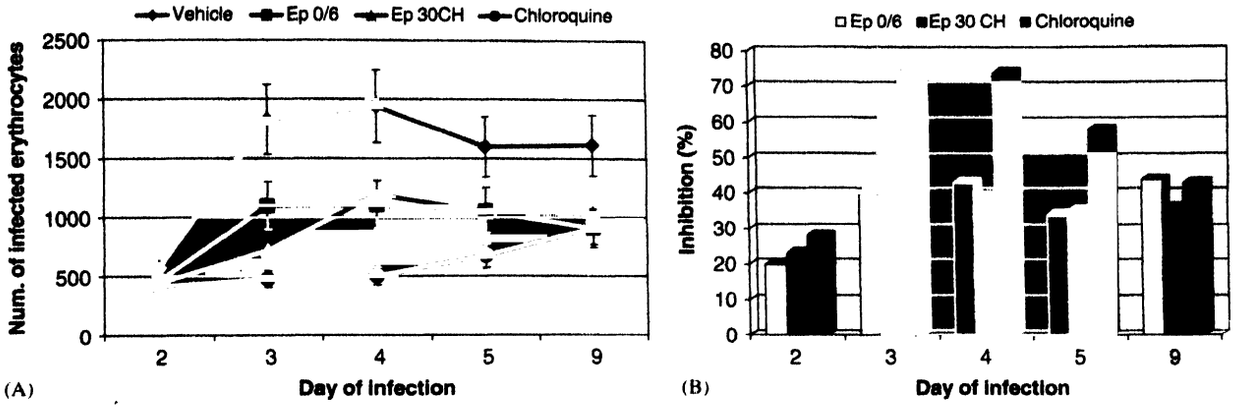

Figure 1 Effect of *Eupatorium perfoliatum* on parasitemia of *Plasmodium berghei* infected mice. BALB/c mice were inoculated i.p. with 10^6 infected erythrocytes and 24 h later were treated orally with *Eupatorium perfoliatum* (0/6 or 30 CH potencies), chloroquine (5 mg/kg/dose) or vehicle, daily over 9 days as described in Materials and Methods. On days 2, 3, 4, 5 and 9 (*X*-axis) a drop of tail blood was obtained from each mouse and used to perform a thin blood smear stained with Giemsa which was observed under a light microscope. (A) Total parasitemia. The number of infected cells in 50 fields were counted and is represented in each point (mean ± SD). Each group consisted of four mice. (B) Inhibitory rate determined using the formula as described in Materials and Methods.

to be infected. Nevertheless, the number of parasites in the mice of this group was always lower than the vehicle-treated group (Figure 1A).

Eupatorium perfoliatum showed an antimalarial effect, but not as marked as for chloroquine, except on day 9 when there was no difference between the treatments. The 30 CH potency showed a slightly more pronounced effect than the 0/6 level in the initial steps of the infection, but from day 4, both potencies showed a similar effect (Figure 1A).

Since to the experiments were conducted with groups of four animals, the results were analysed using the ANOVA test (see Material and Methods). The difference between parasitemia of the control (group treated with vehicle alone) and parasitemia of both groups treated with *Eupatorium perfoliatum* was significant ($P<0.05$) for all the days tested with the exception of day 2.

When parasitemia inhibition rate was calculated using the formula described under Methods, chloroquine treatment showed an inhibition level of 28% on day 2 and 74% on days 3 and 4. After this, the inhibition level fell to 43% by day 9 (Figure 1B). The groups treated with *Eupatorium perfoliatum* showed a similar result to chloroquine, with an inhibition rate of 23% for the 30 CH potency and 20% for the 0/6 potency on day 2, increasing to 61% and 40%, respectively, on day 3. By day 4 the inhibitory capacity stabilized at around 35% and by day 9 the inhibitory rate of the 0/6 potency was similar to that of chloroquine (Figure 1B). On evaluation of parasitemia, on days 4 and 5, an increased number of schizonts were observed in the groups treated with homeopathic preparations of *Eupatorium perfioliatum* with a proportion of 1:2, as compared with the positive and vehicle only controls (Figure 3A). The mice treated with the 0/6 potency level showed a higher number of schizonts than the group treated with the 30 CH potency level, the difference being more noticeable by day 5.

Effect of *Arsenicum album*

Parasitemia in the groups treated with vehicle or chloroquine showed a similar pattern to the *Eupatorium perfoliatum* experiment, however, parasitemia of mice treated with *Arsenicum album* displayed irregular behaviour with a significant antimalarial effect on days 4 and 9, although less so on days 2, 3 and 5 (Figure 2A). The 0/6 potency was always more effective in the control of parasitemia than the 30 CH, especially on day 4 when the number of parasites was similar to the group treated with chloroquine. This effect was also observed on days 5 and 9 but to a lesser extent. However, the statistical difference was significant ($P<0.05$) with respect to the control group treated with vehicle alone (Figure 2A).

When parasitemia inhibitory level was determined, an effect of the 0/6 potency on days 4 and 9 was evident (70% on day 4), and similar to the effect of chloroquine (Figure 2B). The inhibitory effect of the 30 CH potency was always lower. As for *Eupatorium perfoliatum*, the number of schizonts was always higher in the groups treated with *Arsenicum album* than in mice treated with vehicle or chloroquine (Figure 3B) and the group that received the 0/6 potency always displayed the highest value, especially on day 5.

Discussion

We evaluated the effect of homeopathic preparations of *Eupatorium perfoliatum* and *Arsenicum album* on parasitemia of BALB/c mice infected with *Plasmodium berghei*. Because the number of animals used in our experiments was low, we used a statistical test for small samples. A similar number of animals has

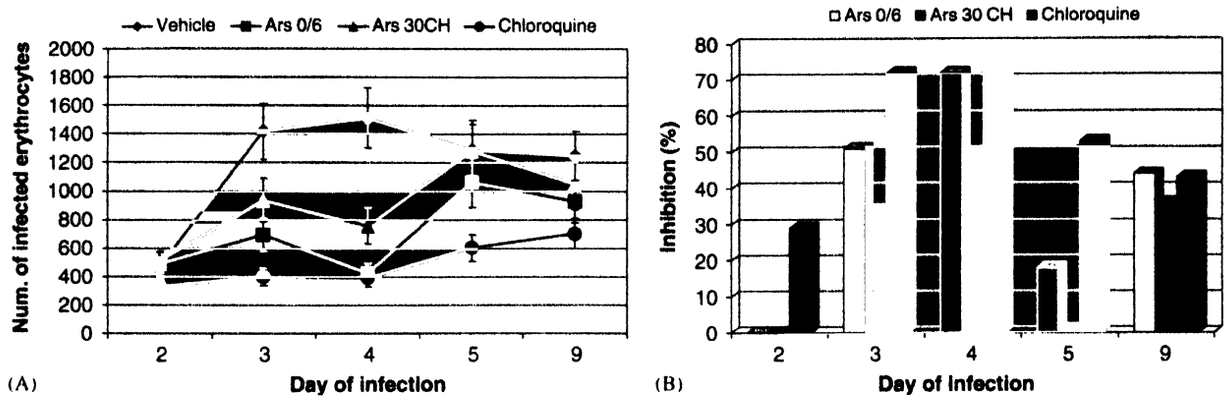

Figure 2 Effect of *Arsenicum album* on parasitemia *of Plasmodium berghei*-infected mice. BALB/c mice were inoculated i.p. with 10^6 infected erythrocytes and 24 h later were treated orally with *Arsenicum album* (0/6 or 30 CH potencies), chloroquine (5 mg/kg) or vehicle, daily over 9 days, as described in Materials and Methods. On days 2, 3, 4, 5 and 9 (*X*-axis) a drop of tail blood was obtained from each mouse and used to perform a thin blood smear stained with Giemsa and observed under a light microscope. (A) Total parasitemia. The number of infected erythrocytes in 50 fields were counted and is represented in each point (mean + SD). Each group consisted of four mice. (B) Inhibitory rate, determined using the formula described in Materials and Methods.

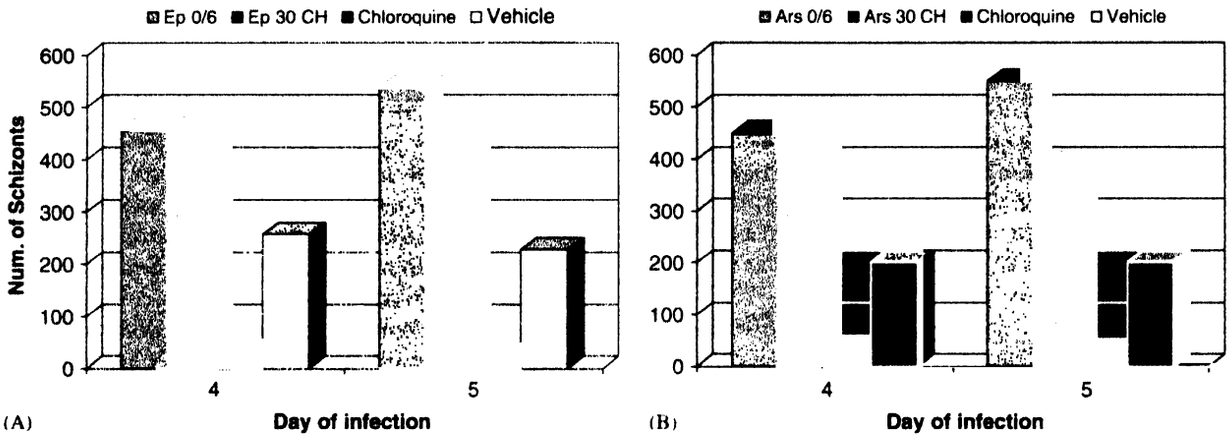

Figure 3 Total schizont count in *Plasmodium berghei* infected mice. The total number of schizonst were counted in thin blood smears stained with Giemsa on days 4 and 5 of infection of each mouse. The mean of total schizonts in each day is indicated (*Y*-axis). (A) Results from mice treated with *Eupatorium perfoliatum*. (B) Result from mice treated with *Arsenicum album*.

been used by other authors in order to evaluate the activity of other antimalarial compounds such as plant extracts,[3,4] 4-anilinoquinoline,[6] Manzamine A[2] and trioxolane.[7]

Eupatorium perfoliatum had a significant antimalarial effect which was stable over several days but not completely inhibitory there were always some infected cells circulating in the blood stream throughout the 9 days of the study. Although the two potencies used were quite different, they showed a similar effects except on day 3, when the 30 CH potency displayed a maximal inhibitory rate (60%). If this result is due to the dilution of the homeopathic drug then this will require more investigation, however, our results are in agreement with other authors in that, although they did not use homeopathic preparations, found an antimalarial effect in extracts of other members of the same genera such as *Eupatorium inulaefolium*[23] and *Eupatorium squalidum*.[24]

With respect to *Arsenicum album*, the effect on parasitemia was very irregular: a significant antimalarial effect being observed on days 3 and 4, but not on day 5. Although it would be useful repeating the assay using a higher number of animals in order to have more conclusive results, the different origin of *Arsenicum album* and *Eupatorium perfoliatum* could explain the different effectiveness of these two medications. In the case of *Arsenicum album* we found a good correlation between the potency and the effect observed; the highest level (0/6) having a marked antimalarial effect at days 3 and 4, with an inhibitory rate of 70%.

Intriguingly, although the effect over parasitemia was different between the two medications, the schizont count was higher in the groups treated with the homeopathic preparations than in the vehicle-treated groups on days 4 and 5, in both cases. At the present we do not have an explanation for this result

but it may be due to treatment delaying this stage of the life cycle of the parasite in some way, possibly by stopping the release of merozoites and interrupting the infection of other erythrocytes. We are not able to comment on mechanism of action of the homeopathic medications tested. *Eupatorium perfoliatum* extracts combined with other plants have shown stimulatory effects on phagocytosis in immune cells[35] and acidic heteroglycans, derived from *Eupatorium perfoliatum* and *Eupatorium cannabium*, have immunostimulatory effects.[36] This opens the interesting possibility that the homeopathic preparation of *Eupatorium perfoliatum* could work in this way rather than having a direct action on the parasite but this will need more investigation.

On the basis of our results we cannot recommend the use of these medications in the treatment of infection caused by *Plasmodium*. But it would be interesting to evaluate their utility as a complement, especially since they have little or no toxicity. The advantages of antimalarial drug combinations have been studied and in several cases result in additive and even synergistic effects have been found.[5,37-41] With all these antecedents it would be interesting to analyse the behaviour of parasitemia of infected animals treated with a combination of the homeopathic medications, such as *Eupatorium perfoliatum* and *Arsenicum album*, along with another antimalarial drug like chloroquine, specially with drug-resistant strains of *Plasmodium*.

Conclusions

In conclusion, homeopathic preparations of *Eupatorium perfoliatum* and *Arsenicum album* had a significant inhibitory effect on parasitemia of BALB/c mice infected with *Plasmodium berghei*. The two potencies used, 0/6 and 30 CH, showed a similar pattern with a maximal inhibitory rate of 60% for *Eupatorium perfoliatum* at 30 CH potency and of 70% for *Arsenicum album* at 0/6 potency. *Arsenicum album* had a more irregular effect on the parasite growth than *Eupatorium perfoliatum*. Finally, the schizont count was higher in the groups treated with the homeopathic preparations than in the vehicle-treated group in both cases.

The experiments were performed with a small number of animals although we were able to perform some statistical analyses for small samples. Additional studies with more animals would be required in order to confirm the results and to enable determination of the effects of different dosage protocols, the mechanism of action and the interactions with other antimalarial drugs. To our knowledge this is the first report on the effect of the homeopathic medications *Eupatorium perfoliatum* and *Arsenicum album* on parasitemia using a rodent malaria model.

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