Evaluation of Antimalarial Activities of the Hydroalcoholic Extracts of Artemisia persica and Artemisia spicigera Against Plasmodium berghei in Albino Mice

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Abstract

Background: Malaria, a life-threatening disease caused by Plasmodium parasites, continues to be a major global health concern. The emergence of drug-resistant strains of Plasmodium highlights the urgent need for new antimalarial agents.

Objectives: This study aimed to evaluate the antimalarial activity of hydroalcoholic extracts obtained from Artemisia persica and Artemisia spicigera against Plasmodium berghei in albino mice.

Materials and Methods: In this study, the hydroalcoholic extracts of the aerial parts of A. spicigera and A. persica were investigated for their effects on parasitemia in mice. Fifty mice were randomly divided into two categories with five groups, with each group receiving either the extract of A. spicigera, A. persica, or a control treatment.

Results: Both extracts of A. persica and A. spicigera inhibited parasitemia on average by 75% and 83.5%, respectively. There was a significant increase in parasitemia at 150 mg/kg of A. persica compared with the negative control group on day 4 (P<0.05). Significant internment of parasitemia was illustrated at 75 mg/kg of A. spicigera in comparison to the negative control group on day 4 (P<0.05).

Conclusion: The findings elucidated that hydroalcoholic extracts of A. persica and A. spicigera plants have the antiplasmodial action to suppress P. berghei infection in mice.

Keywords: Malaria, Chloroquine, Artemisia persica, Artemisia spicigera, Antimalaria activity, Plasmodium berghei

Background

Malaria is an important parasitic disease that affects many countries, and 3.2 billion people are at risk of this disease in the world.1 Plasmodium multiplies and develops in the gut of the Anopheles mosquito, then enters the body of the vertebrate host, including humans, through the bite of an infected Anopheles mosquito, and completes its life cycle in the liver and red blood cells, respectively.2 In 2020, malaria killed more than 600,000 people worldwide.3 In Iran, the transmission of Plasmodium vivax and Plasmodium falciparum predominantly occurs in the southeastern region, which shares borders with Pakistan and Afghanistan.4 One of the major challenges in combating malaria is the emergence of drug-resistant strains of P. falciparum, posing a significant problem in many countries. Additionally, the control and treatment of malaria have become increasingly complex due to the widespread resistance of Plasmodium species to commonly available antimalarial drugs such as chloroquine, mefloquine, and sulfadoxine-pyrimethamine.5,6 Consequently, there has been a growing interest in exploring alternative drugs, particularly the use of plant-derived antimalarial medicines. The Compositae family, which comprises over 1000 genera and 20,000 species, has garnered attention in this regard.7 In
this family, the genus *Artemisia* has demonstrated various beneficial properties, including antimicrobial, antioxidant, anticytotoxic, anti-inflammatory, insecticidal, and repellent effects. Artemisinin, a compound isolated from the plant *Artemisia annua*, has emerged as the most effective herbal antimalarial treatment for drug-resistant malaria worldwide. However, the emergence of artemisinin-resistant *P. falciparum* strains in Southeast Asia has necessitated further research to identify new compounds for malaria treatment. Some species of the genus *Artemisia* have exhibited various beneficial properties, including antimalarial activity. In Iran, there are two species of *Artemisia*, namely, *Artemisia spicigera* and *Artemisia persica*, locally known as Derme ye sonbolei and Persian Derme. Despite their potential anti-malarial role, the effect of these species has not been studied on *Plasmodium*. *P. berghei* is a suitable model for animal studies. Therefore, this study sought to investigate the antimalarial activity of hydroalcoholic extracts derived from *A. persica* and *A. spicigera* against *P. berghei* in albino mice.

**Materials and Methods**

**Plant Material**

*Artemisia persica* and *A. spicigera* aerial parts were prepared and identified, and voucher specimens were deposited at the Herbarium of the Iranian Biological Resource Center, Tehran, Iran. The aerial parts of these plants were dried at room temperature and then powdered by a mixer.

**Plant Extraction**

*Artemisia spicigera* and *A. persica* (20 g) aerial parts were put in percolators and macerated in 200 mL of ethanol (Merck) 80% (3 × 48). The achieved extracts were filtered through Whatman (no1, diameter 150 nm, England), and solvents were evaporated by a Rotary evaporator (Heidolph, Germany) at 37 °C. Two extracts were stored at 4 °C for further investigation. Subsequently, three concentrations, including 25, 75, and 150 mg/mL, were prepared.

**Animals and Parasite Strain**

The study was conducted at the medical school animal house at Alborz University of Medical Sciences, focusing on female Swiss albino mice aged 4–6 weeks and weighing 25 ± 5 g. These mice were purchased from the Laboratory Animal Department, Karaj Production and Research Complex, Razi Institute of Iran, and were housed in the animal house of the medical school at Alborz University of Medical Sciences. Before the study initiation, the mice were given a week to adapt to laboratory conditions. To investigate the anti-malarial properties in vivo, the researchers used a chloroquine-sensitive strain of *P. berghei* (ANKA strain), which was obtained from the Medical Parasitology Laboratory at Tehran University of Medical Sciences. The parasites were maintained through the serial passage of blood from infected mice with a parasitemia level of 20%–30% to non-infected mice. This study aimed to elucidate the anti-malarial effects in mice using the aforementioned strain of *P. berghei*. The findings from this research can contribute to our understanding of potential treatments for malaria.

**Parasite Insemination**

The mice infected with *P. berghei* were utilized as donor animals for this study. To obtain the infected red blood cells, a cardiac puncture technique was employed, with ketamine and xylazine administered as anesthesia drugs. The resulting erythrocytes were then diluted in a physiological saline solution (0.9%). The dilution contained 5 × 10⁷ diseased erythrocytes in 1 mL of blood. Subsequently, each mouse received an intraperitoneal injection of a blood suspension (0.2 mL) containing 1 × 10⁷ parasitized erythrocytes.

**Toxicity Assay of Extracts in Naïve Mice**

Mice were distributed into two categories with five groups (n = 5 mice/group), including groups 1 (low dose), 2 (average dose), 3 (high dose), 4 (negative control), and 5 (positive control). Then, three different concentrations per extract, including 25, 75, and 150 mg/mL, were used for their toxicity effects. All groups were injected with 200 µL of related solutions by intraperitoneal injection (IP) once a day for 7 days.

**In Vivo Antimalarial Activity**

The antimalarial activity was elucidated by the 4-day suppressive standard test. The mice were randomly distributed into two categories for each extract, with five groups (n = 5 mice/group). All mice were infested with *P. berghei* on the first day (D0). Treatment began 3 hours after pollution on day 0 and then continued daily for four days (i.e., from day 0 to day 3). All treatment groups received 0.2 mL of solutions (*A. spicigera* and *A. persica*) with different dosages, including groups I (25 mg/kg), II (75 mg/kg), III (150 mg/kg), and IV (positive control), which received the standard antimalarial drug (chloroquine) 20 mg/kg with the same amount of volume (0.2 mL), and Group V (negative control), which received normal saline with the same amount of volume (0.2 mL) daily for 4 days. The mice received extracts and the standard drug through IP. On days 5 (D4), 8 (D7), 12 (D11), and 16 (D15), blood samples were collected from the tail snip of each mouse. The smears were stained with a 20% Giemsa solution. The slides were examined under an optical microscope. The parasitaemia was controlled by counting a minimum of 5 different fields per slide (each of approximately 200 RBCs).
Cell volume quantity was measured before infection on days 0 and 4. Blood was gathered from the tail of each mouse and filled in heparinized capillary tubes. It was centrifuged by centrifuge (Hettich, Germany, 12,000 rpm) for 5 minutes and then measured. The survival rate of mice was determined daily. The number of treatment days was recorded for each mouse, along with the control groups.

**Analysis**

Data were studied using the computer software SPSS, version 21. The outcomes of the study were expressed as a mean ± standard error of the mean (M ± SEM). Statistical significance was determined by one-way analysis of variance with multiple comparison tests (Post Hoc/Tukey’s test/HSD) to compare parameters within groups.

**Results**

The efficacy of hydroalcoholic extracts of *A. persica* and *A. spicigera* inhibited parasitaemia on average by 75% and 83.5%, respectively. Hydroalcoholic extracts of *A. persica* and *A. spicigera* showed the best chemo-suppressive effect in mice diseased with the *P. berghei* parasite on day 4. Table 1 provides the parasitaemia suppressive effect of different doses of the hydroalcoholic extract of *A. persica* and *A. spicigera* on D4, D7, D11, and D15. There was substantial suppression of parasitaemia at 150 mg/kg of *A. persica* compared to the negative control group on day 4 (P < 0.05, Figures 1 and 2). However, there was a suppression of parasitaemia at 75 mg/kg of the *A. persica* extract compared to all tested doses (P < 0.05). There was a parasitaemia suppressive effect by different doses of the hydroalcoholic extract of *A. spicigera* on D4, D7, D11, and D15 (Figure 3). Significant suppression of parasitaemia (P < 0.05) was found at 75 mg/kg of *A. spicigera* compared to the negative control group on day 4 (Figure 4). In addition, significant suppression of parasitemia was detected at 150 mg/kg of this extract in comparison to the negative control group.

![Figure 1. Effects of Different Doses of the Hydroalcoholic Extract of Artemisia persica on Parasitemia on the Fourth Day](image)

**Table 1. Effects of Different Doses of Hydroalcoholic Extracts of Aerial Parts of Artemisia persica and Artemisia spicigera on Parasitemia**

<table>
<thead>
<tr>
<th></th>
<th><em>Artemisia persica</em></th>
<th><em>Artemisia spicigera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No.</strong></td>
<td><strong>Dose Mg/Kg</strong></td>
<td><strong>Mean of Parasitaemia</strong></td>
</tr>
<tr>
<td><strong>D4</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>8.8</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>7.7</td>
</tr>
<tr>
<td>5</td>
<td>Normal saline</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Chloroquine</td>
<td>0</td>
</tr>
<tr>
<td><strong>D7</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>14.6</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>9.8</td>
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<tr>
<td>5</td>
<td>150</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>Normal saline</td>
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<tr>
<td>5</td>
<td>Chloroquine</td>
<td>0.00</td>
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<tr>
<td><strong>D11</strong></td>
<td></td>
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<tr>
<td>5</td>
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<tr>
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<td>Normal saline</td>
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<td>5</td>
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<tr>
<td><strong>D15</strong></td>
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<tr>
<td>5</td>
<td>Chloroquine</td>
<td>0.00</td>
</tr>
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Note: No.: Number; Max.: Maximum; Min.: Minimum.
Najm et al. effect on the growth of murine malaria after day 4 post-infection. The results of the present study also revealed no signs of toxicity or mortality in all treated mice, even with high concentrations of the extracts. Studying the effect of two plants from the genus *Artemisia* that grow naturally in Iran on *P. berghei* for the first time to find an effective drug for the treatment of malaria, one of the indigenous diseases of Iran, was one of the strengths of this research. The outcomes of our study are in line with the findings of studies on two other *Artemisia* species, including *A. nilagirica* and *A. vulgaris* leaf extracts, and studies on leaf crude extracts of *Maytenus gracilipes* and *Eucalyptus globulus* Labill that suppressed parasitemia in infected mice with *P. berghei*.22-25 *A. spicigera* generally contains total phenols and flavonoids.26 It seems that these substances are involved in the antimalarial effect of the *A. spicigera* extract. Our study, similar to a study on the ability of *Vernonia amygdalina-A. annua* combination as an herbal artemisinin combination treatment of murine malaria and a study on the effect of *Alstonia boonei* and *Carcia papaya* against *P. berghei*, could significantly increase the survival rate of mice compared to the negative control group.27,28 However, the parasitaemia was reduced by increasing the concentration of the extracts. This suggests that isolating potent fractions can be more effective in suppressing parasitaemia. The extracts investigated in the present study, similar to the studies on *Senna occidentalis* methanolic root, *Lagenaria siceraria*, and *Otostegia persica* extracts, had safety and significant effectiveness in inhibiting *P. berghei*.29,30,31 One of the limitations of our study was that we could not access the different fractions of the extracts, and consequently, we could not measure the safety and

negative control group on day 7 (*P* < 0.05). Both extracts were able to increase the survival time of mice compared to the negative control group.

**Discussion**

Many efforts are being made to develop new drugs against malaria, but still, resistance to malaria drugs is spreading around the world, and malaria causes the deaths of thousands of people annually. Therefore, to overcome drug resistance, several studies have been conducted on herbal compounds, including the *Artemisia* genus and its compounds. Antimalarial natural compounds such as sesquiterpenes, steroids, flavonoids, alkaloids, stilbenes, diterpenes, and coumarin derivatives are present in some species of the *Artemisia* genus.21 Our study showed that both *A. persica* and *A. spicigera* species have an inhibitory effect on the growth of murine malaria after day 4 post-infection. The results of the present study also revealed no signs of toxicity or mortality in all treated mice, even with high concentrations of the extracts. Studying the effect of two plants from the genus *Artemisia* that grow naturally in Iran on *P. berghei* for the first time to find an effective drug for the treatment of malaria, one of the indigenous diseases of Iran, was one of the strengths of this research. The outcomes of our study are in line with the findings of studies on two other *Artemisia* species, including *A. nilagirica* and *A. vulgaris* leaf extracts, and studies on leaf crude extracts of *Maytenus gracilipes* and *Eucalyptus globulus* Labill that suppressed parasitemia in infected mice with *P. berghei*.22-25 *A. spicigera* generally contains total phenols and flavonoids.26 It seems that these substances are involved in the antimalarial effect of the *A. spicigera* extract. Our study, similar to a study on the ability of *Vernonia amygdalina-A. annua* combination as an herbal artemisinin combination treatment of murine malaria and a study on the effect of *Alstonia boonei* and *Carcia papaya* against *P. berghei*, could significantly increase the survival rate of mice compared to the negative control group.27,28 However, the parasitaemia was reduced by increasing the concentration of the extracts. This suggests that isolating potent fractions can be more effective in suppressing parasitaemia. The extracts investigated in the present study, similar to the studies on *Senna occidentalis* methanolic root, *Lagenaria siceraria*, and *Otostegia persica* extracts, had safety and significant effectiveness in inhibiting *P. berghei*.29,30,31 One of the limitations of our study was that we could not access the different fractions of the extracts, and consequently, we could not measure the safety and
efficacy of fractions separately.

### Conclusion

This study elucidated that both extract plants have antiplasmodial activity to suppress *P. berghei* infection in mice. Further investigation of these plants is needed for the isolation and identification of active compounds that are responsible for the antimalarial action of these plants.

### Acknowledgments

We are most grateful to the staff of the animal house for their cooperation.

### Competing Interests

The authors express that there is no conflict of interests regarding the publication of this paper.

### Ethical Approval

This study was approved by the Research Ethical Review Committee of Alborz University of Medical Sciences, Karaj, Iran (with approval number: Abzums.Rec.1396.58).

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