

**Pharmacochemistry of Iranian Flora *Artemisia*
oliveriana and its Antimalarial Effects on
Plasmodium berghei in Vivo**

**Touraj Miandoabi¹, Khosrow Hazrati Tappeh^{1,*}, Hossein Nahrevanian^{2,*},
Seyed Masoud Kazemi³, Habib Mohammadzadeh¹ and Haleh Hanifian¹**

¹ Department of Parasitology & Mycology
Urmia University of Medical Sciences Urmia, Iran

² Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran

³ Department of Applied Chemistry, Islamic Azad University
Qom Branch, Qom, Iran

* Corresponding author

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Abstract

The aim of this study was to evaluate antimalarial effects of Iranian flora *Artemisia oliveriana* on *Plasmodium berghei* in vivo and photochemistry of its natural effective components. This is the first application of Iranian flora *A. oliveriana* on murine malaria in Iran. The aerial parts of *A. oliveriana* were collected at the flowering stage from Qum and Isfahan provinces, in central part of Iran in 2010. The aerial parts were air-dried at room temperature and then powdered and macerated in methanol and filtered and it was extracted and dried by Rotary Evaporator. The toxicity of herbal extract was assessed on four groups of naive NMRI mice and its antimalarial efficacy was investigated on infected *P. berghei* animals. The significance of differences was determined by student's t-test using Graph Pad prism software. The results indicated no toxicity was observed even by high concentration of herbal extract by measuring body weight, survival rate and hepato/ splenomegaly. It is demonstrated that total extract *A. oliveriana* possesses therapeutic inactivity against *P. berghei* which indicates its antimalarial effects in vivo on NMRI mice.

Keywords: *Artemisia oliveriana*; antimalaria; *Plasmodium berghei*; Malaria; Herbal extract

Introduction

Malaria is one of the oldest and most perilous diseases which encountered by mankind ever [1]. This infectious disease causes by *plasmodium* parasites transmitted by female anopheles mosquitos in malaria's regions. The causing agents are 4 species of Plasmodium included *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*. Among these species *P. falciparum* is the cause of malignant and life threatening tertiary malaria. 300-500 million clinically cases and 1-2 million death reports as incidence of malaria in the world [2]. In severe malaria, death may occur within 24 h or less than a day [3]. Since the production of an effective vaccine for malaria is still a major problem, chemotherapy remains as best weapon in fighting against malaria. However the resistance to the current limited antimalarial drugs and emergence of multi-resistant strains are the main issues which clear urgent need for new anti-malarial compounds.

Resistance to all classes of antimalarial drugs has reported except artemisinin. Plant-derived artemisinin is currently the only available drug acts effectively. The most significant recent investigation in natural antimalarial drugs is undoubtedly the identification of artemisinin as the active component of the plant *Artemisia annua*, The Chinese traditional medicine as an antimalarial agent. *Artemisia* possesses a unique active part identified as sesquiterpene which contains endoperoxide group that is the most important part of product. Artemisinin is an effective anti-malarial drug with significant activities in chloroquine resistant strains. It is a natural product, which considered as important agent against the parasite with low toxicity during treatment process of malaria disease [2, 4-6]. The aim of this study was to evaluate Iranian flora *Artemisia oliveriana* as a natural component for its antimalarial effects against *Plasmodium berghei* in vivo. This is the first report on *A. oliveriana* extract as flora from the Qom Province, for treatment of murine malaria on *P. berghei* infected NMRI mice.

Materials and methods

Animals

Female outbreed NMRI (Naval Medical Research Institute) mice supplied by the Laboratory Animal Department, Karaj Production and Research Complex, Pasteur Institute of Iran.

Plant samples

The aerial parts of Iranian flora *A. oliveriana* were collected at flowering stage from Qum and Isfahan provinces, in central part of Iran in October 2010. Type, genus and specie were identified at the Herbarium of the Research Institute of Forests and Rangelands (RIFR), Tehran, Iran. After identification, specimens were

air-dried at room temperature under shade and ground into a fine powder using an electric grinder. The powdered samples were stored in a cold room (4 °C) in appropriate containers.

Herbal extraction

The aerial parts were air-dried at room temperature then were powdered by mixer. The powder (248 gr) of *A. oliveriana* was macerated in 1.5 lit methanol (Merck) and then kept for 72 h away from light and high temperature. It was filtered, evaporated and dried by Rotary evaporator (Eyela, N-1000, Japan) and finally defatted in refrigerator. Wet weight of raw extract at the final step was 20.3 gr and its color was dark green. The extract was kept in refrigerator until applied for the toxicity assay.

Toxicity assay of herbal extract in naïve mice

In vivo toxicity was evaluated by using total extract on naïve mice. One gram of herbal extract was dissolved in vehicle (ethanol 60% with normal saline) using a thermal stirrer Cenco, Netherland) to achieve homogenate suspension. The mice were divided into 4 groups (n=10), including Group 1 (control), Group 2 (10 mg/kg bw), Group 3 (100 mg/kg bw), group 4 (1000 mg/kg bw). Subsequently three concentrations (low, average, high dose) including 1, 10, 100 mg/ml were prepared. The mice were inoculated with 0.2 ml of related solutions; the control group was inoculated with vehicle [subcutaneously (sc) once a day for 8 days].

Malaria Parasites

Chloroquine sensitive strain of *P. berghei* was stored in liquid nitrogen in Urmia University of Medical Sciences, Medical Parasitology Laboratory and maintained by blood passage in mice.

Inoculation of malaria parasites

Mice were inoculated (0.2 ml) intravenously (iv) into a tail vein with blood from a *P. berghei* infected donor mouse to contain 2×10^5 parasitised red blood cells (PRBC).

Anti-malarial effects of total extract on malarial mice

The highest dose of herbal extract (100 mg/ml) was selected to evaluate its antimalarial activity on malaria mice. Animals were divided into two groups (n=10 mice/group), including control and test; both groups were infected with *P. berghei*. Herbal extract were injected into test group and control group received vehicle (0.2 ml, sc, once a day for 2 weeks).

Assessment of Pathology

Parasitaemia

Parasitaemia was determined on different days after infection using blood smears stained with Geimsa (Sigma Co., India). PRBC were counted in five different fields, each of approximately 200 cells. Results are expressed as the mean percentage

(%) of erythrocytes containing Geimsa positive bodies. Experiments were licensed under the Animals (Scientific Procedures) Act 1986. In compliance with the conditions of this license, infected animals were humanely killed at the onset of the terminal phase of malaria.

Degree of hepato/splenomegaly

Entire livers and spleens were removed post mortem at the end of the experimental period from mice after induction of terminal general anesthesia by inhalation of diethyl ether (Merck, India). Organ wet weights were measured as indices for degree of hepato/splenomegaly.

Body weight

Body weight was measured initially at different days 1, 14 of experiment, using a top pan balance (OHAUS Scale Corp., USA) as a major indication of pathology.

Measurement of survival rate

Survival rate was presented as the percentage of surviving experimental mice at every other week after inoculation and compared with appropriate vehicle-treated control group.

Statistical analysis

The results were expressed as (M±SEM) (mean ± Standard error of the mean). The significance of differences was determined by analysis of variances (ANOVA) and Student's *t*-test, using the Graph Pad Prism (Graph Pad, San Diego, California, USA).

Results

Toxicity assay in naïve mice

The results presented no toxicity which was observed *in vivo* even with high dose of *A. oliveriana* extract. Pathophysiological signs including splenomegaly, hepatomegaly, body weight and survival rate represented no side effects of total extract (Figure 1).

Antimalarial effects of total extract in malaria mice

A. oliveriana significantly decreased parasitaemia after day 9 post-infection (Figure 2). No side effects on pathophysiology represented by total extract in malarial mice (Figure 3).

Discussion

Since producing effective anti-malarial vaccines is still complicated, chemotherapy remains the first-line action against the disease; however at present, the medicinal treatment of malaria has faced with drug resistance which is key problem in some malaria endemic areas of Iran [2, 7].

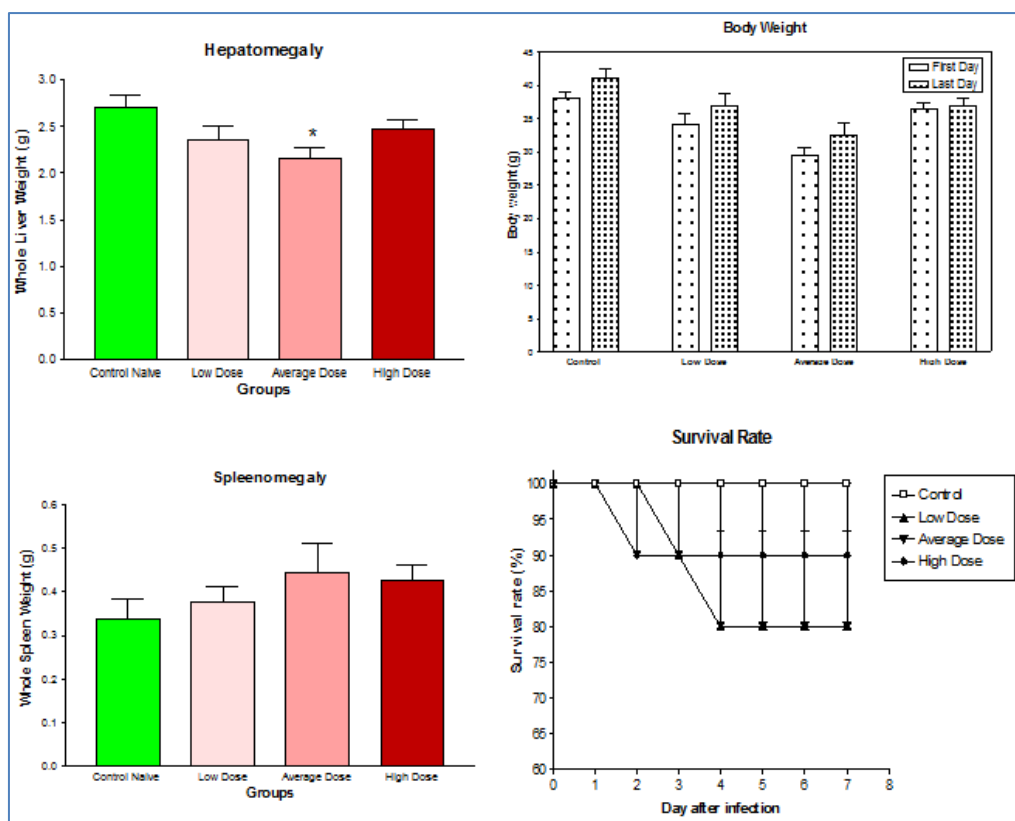


Figure 1: Physiopathology of Iranian flora *Artemisia oliveriana* on naive mice

In chemotherapeutic agents, issue of resistance means that searching for new antimalarial drugs is an urgent priority. In addition to the need for the development of new antimalarial drugs, it is important to prove the efficacy and safety of natural agents of traditional medicinal plants using against malaria [4]. It is well-known that application of traditional medicine against malaria in some endemic countries with lack of access to essential medicines play very important role in treatment [8]. Like most natural based medicines, artemisinin exists in the *Artemisia* plants in very low-doses concentration. Various species of the genus *Artemisia* are used for their pharmacological, antimicrobial, antioxidant activity in world and Iran [9, 10]; however this is the first report on application of *A. oliveriana* extract on the treatment of murine malaria in vivo.

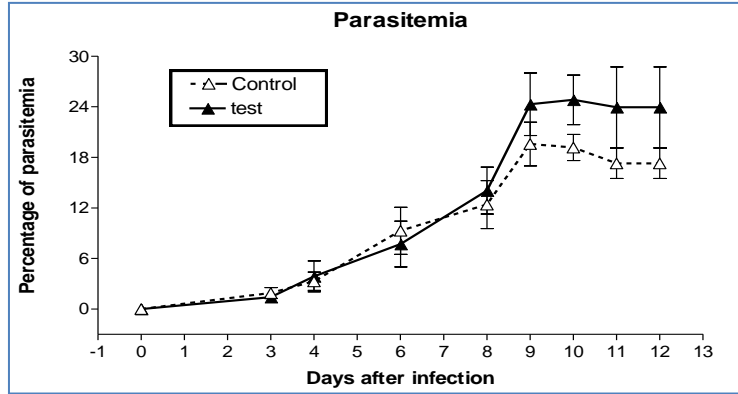


Figure 2: Antimalarial effects of Iranian flora *Artemisia oliveriana* on *Plasmodium berghei* infected mice

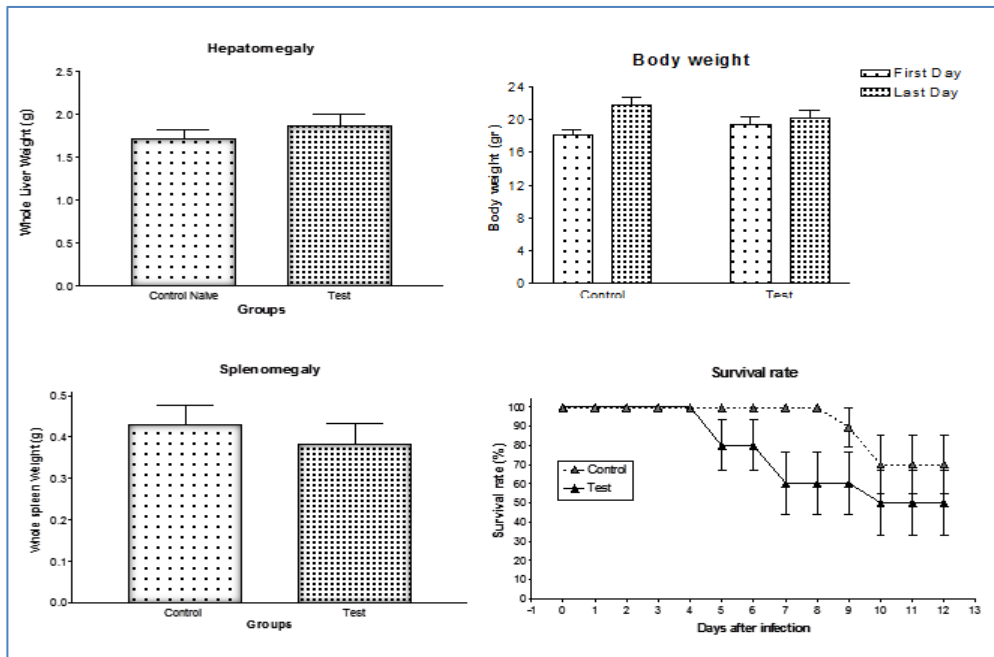


Figure 3: Physiopathology of Iranian flora *Artemisia oliveriana* on *Plasmodium berghei* infected mice

In addition to previous publications, the herbal extract of Iranian Flora *A. khorassanica* was successfully tested in vivo for its antiplasmodial activity through artemisinin composition, which is globally used as a standard malaria treatment [2]. Similar studies establish the efficacy of natural agents and traditional remedies such as *Boerhaviaelegans*, *Solanumsurattense*, *Phyllanthusama-*

rus and *Osyrisquadripartite* [1-4]. Some chemical extract of the aerial parts of *A. diffusa* in the Province of Khorassan (Iran) showed its antimalarial activity [11]. The genus *Artemisia* has always been of great botanical and pharmaceutical interest and is useful in traditional medicines for a treatment of the variety of diseases and complaints [12, 13]. Moreover, Rustaiyan *et al.*, reported the effects of *A. diffusa* crude extracts and the fractions of plant including Tehranolide, on the developmental stages of *P. berghei* by decreasing parasitaemia [14]. Antimalarial effects of different Iranian flora of *Artemisia* herbal extracts including *A. turanica*, *A. absinthium*, and their effective agent (Tehranolide) against malaria were successfully evaluated [15, 16].

Although, the current study evaluated the anti-malarial activity of the *A. oliveriana* extract on *P. berghei*, however the microscopic examination of Giemsa-stained slides did not showed a significant inhibitory effect on stage of the murine malaria treated with these herbal extracts. Moreover, this study confirmed some efficacies of this extract on pathophysiological symptoms of murine malaria by this agent. Conclusively, the *A. oliveriana* extract did not show significant antimalarial effects against murine malaria *in vivo*. There is requirement to find the major component of this herbal extract by further studies. More investigations are recommended on different Plasmodia and animal hosts to better clarify anti-malarial activity of Iranian flora *A. oliveriana* and analysis of its natural components.

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