



Phytochemical evaluation and antimalarial effects of *Artemisia turanica* herbal extracts as an Iranian flora on *Plasmodium berghei* in vivo

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Abstract

The aim of this study was to evaluate the therapeutic effects of Iranian flora *Artemisia turanica* against *Plasmodium berghei* in vivo and antimalarial evaluation of its different extracts. This is the first application of Iranian flora *A. turanica* herbal extracts on treatment of murine malaria. The aerial parts of *A. turanica* were collected at flowering season from Bojnord, north eastern Iran in 2009. They were air-dried at room temperature; powder was macerated in methanol; suspension was filtered and it was extracted and dried by Rotary Evaporator. Total herbal extract was subsequently processed to prepare ether and chloroform extracts. The toxicity of herbal extract was assessed on naïve 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 *A. turanica* possesses therapeutic activity against *P. berghei*. Total extract presented antimalarial effects against *P. berghei* and can be attributed to the presence of its compounds as an effective therapy.

Key words : *Artemisia turanica*; antimalaria; *Plasmodium berghei*

1. Introduction

Malaria is one of the world wide parasitic diseases which threaten life of more than hundred million people at the malarial areas each year ^[1]. The cause of malaria is a protozoa called *Plasmodium*, which is transmitted by anopheles

mosquitoes. Malaria is found throughout the tropical and sub-tropical regions of the world and causes more that 300 million acute illnesses and at least one million deaths annually ^[2]. Although among the four human malaria

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parasites, *Plasmodium falciparum* causes the most severe forms of disease, however the number of reported cases of *P. vivax* has been increasing in many endemic regions [3-4]. After transmission, sporozoites enter a blood vessel at the bite site and transfer via the blood stream to the liver as their initial site of replication in host [5].

Although attempts for malaria control are extensively conducted at the malarial areas, malaria still remains a problem due to drug resistance, limitation of vector control and lack of suitable vaccine [6-7]. However, diagnosis and treatment are the main interventions of the global malaria control strategy [8-9]. Monitoring drug efficacy is important for the impact of therapy and drug policy. The most important antimalarial drugs including Artemisinin, Chloroquine, Quinine, Mefloquine and Fansidar have their own challenges [10]. However, their availability and cost [11], side effects and drug resistance are major problems [12-13]. Therefore there is great need for the development of effective and safe drugs for malaria [14]. A number of drugs have originated from plant sources, and the potential of plants for the production of new antiprotozoal agents has recently been emphasized [15-17]. Artemisinin is the most important antimalarial drug, which is derived from sweet worm wood, *Artemisia annua*. Artemisinin is a sesquiterpene lactone containing an endoperoxide bridge that is considered essential to its antimalarial action killing asexual stages, as well as gametocytes [18-22]. Artemisinin derivatives including arteminic acid, artemether, artemotil, artemimol and artesunate were effective against many types of malaria [23-25].

The genus *Artemisia* has always been of great pharmaceutical interest for a treatment of the variety of diseases [26-28]. *A. annua* is presently being cultivated on a commercial scale for its antimalarial sesquiterpene lactone. The genus is

of small herbs and belongs to the important family Compositae (Asteraceae), which comprises about 1000 genera and over 20,000 species. *Artemisia* is found in Europe and North America but mainly is dominating the Asia [29-31]. Among the Asian *Artemisia* flora, 150 species were recorded for China, 50 species reported to occur in Japan and 34 species of the genus are found in Iran, of which may be endemic; *A. melanolepis* Boiss and *A. kermanensis* Pold[32], *A. absinthium* [33], *A. annua* [34], *A. dracuncululus*[35], *A. aucheri* [36], *A. haussknechtii* Boiss [37], *A. scoparia*, *A. sieberi* [38] and *A. sieberi* Besser [39].

Pharmacology of different genus of Iranian *Artemisia* species has been studied and the presence of variety of components including monoterpenes[40], sesquiterpenes[41,42], sesquiterpene lactones [43,44] and essential oils [45,46] were fully reported [33-36]. The aim of this study was to evaluate Iranian flora *A. turanica* for its antimalarial effects against *Plasmodium berghei* *in vivo* and phytochemical analysis of its different extracts. This is the first report on application of *A. turanica* extracts as Iranian flora for treatment of *Plasmodium berghei* in malarial mice.

2. Materials and methods

2.1 Plant samples

The aerial parts of Iranian flora *A. turanica* were collected at flowering season from Bojnord in Khorasan province, north-eastern Iran in 2009. Type, genus and species was identified at the Herbarium of the Research Institute of Forests and Rangelands (RIFR), Tehran, Iran.

2.2 Animals

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

Institute of Iran) were used in this investigation. The mice were kept at room temperature (20- 25 °C) on a 12h light and 12h dark cycle, with adequate food and water. Experiments with animals were done in accordance with the NIH guidelines, and measures taken to protect animals from pain or discomfort. It has been approved by Ethical Committee of the Pasteur Institute of Iran, in which the work was done.

2.3 Herbal extraction

The aerial parts were air-dried at room temperature then were powdered by mixer. The powder (140gr) of *A. turanica* was macerated in 1 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 13.3 gr and its color was dark green. The extract was kept in refrigerator until applied for the toxicity assay.

2.4 Toxicity assay

Toxicity was evaluated by using total extract on naïve mice. One gram of herbal extract was dissolved in vehicle (9 ml ethanol + 1 ml normal saline) using a thermal stirrer (Cenco, Netherland) to achieve a homogenate suspension. The mice were divide into 4 groups (n=5), 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 7 days].

2.5 Malaria parasite

Murine malaria parasite *Plasmodium berghei* NY

was kindly donated by Dr. M. J. Dascombe from the School of Life Sciences, University of Manchester, UK. Malaria parasite was maintained by blood passage in NMRI mice when active parasites were required; otherwise it was stored at -70°C in Alserver's solution (2.33% glucose, 0.525% NaCl and 1% sodium citrate in deionised water) and glycerol (9:1 parts by volume).

2.6 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 2×10^6 parasitised red blood cells (PRBC).

2.7 Antimalarial effects of total extract on malarial mice

The results of toxicity assay did not represent any toxicity even by the highest dose of herbal extract; therefore 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 1 month).

2.8 Ether and chloroform extraction of *A. turanica* compounds

Herbal extract was eluted with 300 ml n-hexane (Sigma, Co. India), two phases were separated, the lower hexane phase (non-polar compounds) was collected and kept at refrigerator for further experiment. The upper phase was eluted with 300 ml chloroform (Merck, India) 3 times; subsequently lower chloroform phase was collected, evaporated and extracted. Higher methanol phase was then eluted with 300 ml diethyl ether (Merck, India) 3 times. Finally, ether phase was collected, evaporated and

extracted. It is suggested semi-polar components could be separated in these two chloroform and ether phases. The extracts were kept in refrigerator until used for injection in mice.

2.9 Antimalarial effects of ether and chloroform extracts of *A. turanica*

In addition to total extract, the ether and chloroform extracts were applied for their antimalarial activity. Animals were divided into 4 groups ($n=5$) including control and test groups for each extract. Entire groups were infected with murine malaria parasite and injected with 0.2 ml of extract (test) and vehicle (control) sc, once a day for 2 weeks.

2.10 Assessment of Pathology

2.10.1 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.

2.10.2 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 anaesthesia by inhalation of diethyl ether (Merck, India). Organ wet weights were measured as indices for degree of hepatomegaly and splenomegaly.

2.10.3 Body weight

Body weight was measured initially at different days 1, 7, 21 of experiment, using a top pan

balance (OHAUS Scale Corp., USA) as a major indication of pathology.

2.10.4 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.

2.10.5 Statistical analysis

Values are presented as the mean \pm SEM for groups of n samples. The significance of differences was determined by ANOVA and Student's t -tests using Graph Pad Prism Software (Graph Pad, San Diego, California, USA).

3. Results

3.1 Toxicity assay in naïve mice

The results presents no toxicity was observed *in vivo* even with high dose of *A. turanica* total extract. Pathophysiological signs including splenomegaly, hepatomegaly, body weight and survival rate represented no side effects of total extract.

3.2 Antimalarial effects of total extract in malaria mice

The results indicated efficacy of total extract on reducing parasitaemia from $24.6 \pm 2.5\%$ to $7.6 \pm 0.7\%$ (Figure 1). No side effects on pathophysiology represented by total extract in malarial mice (Figure 2).

3.3 Antimalarial effects of ether and chloroform extracts in malaria mice

The inhibitory effects of the *A. turanica* ether and chloroform extracts on malaria were observed by reduction the parasitaemia (Figure 3). No pathophysiological changes were indicated after treatment in test groups when compared with those in control groups (Figure 4).

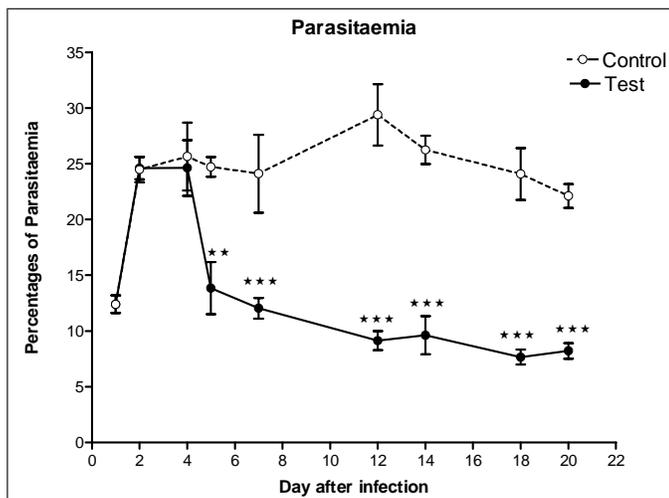


Figure 1: The effects of *A. turanica* extract on parasitaemia during malaria infection in mice Control (drug vehicle); Test (Total extract of *A. turanica*; n=10 mice/group/day, Statistical analysis using Student's *t*-test, P values: ** P<0.01, *** P<0.001).

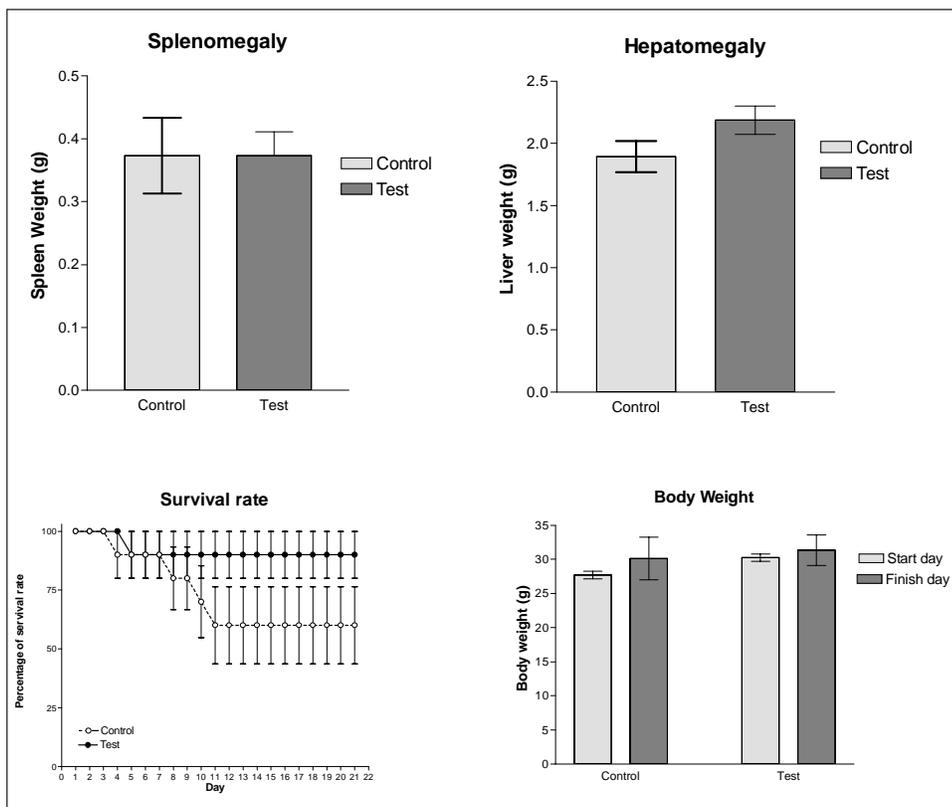


Figure 2: Pathophysiological evaluation of *A. turanica* total extracts injection in malaria mice Control: drug vehicle; Test: Total extract; n=10 mice/day/group, Student's *t*-test

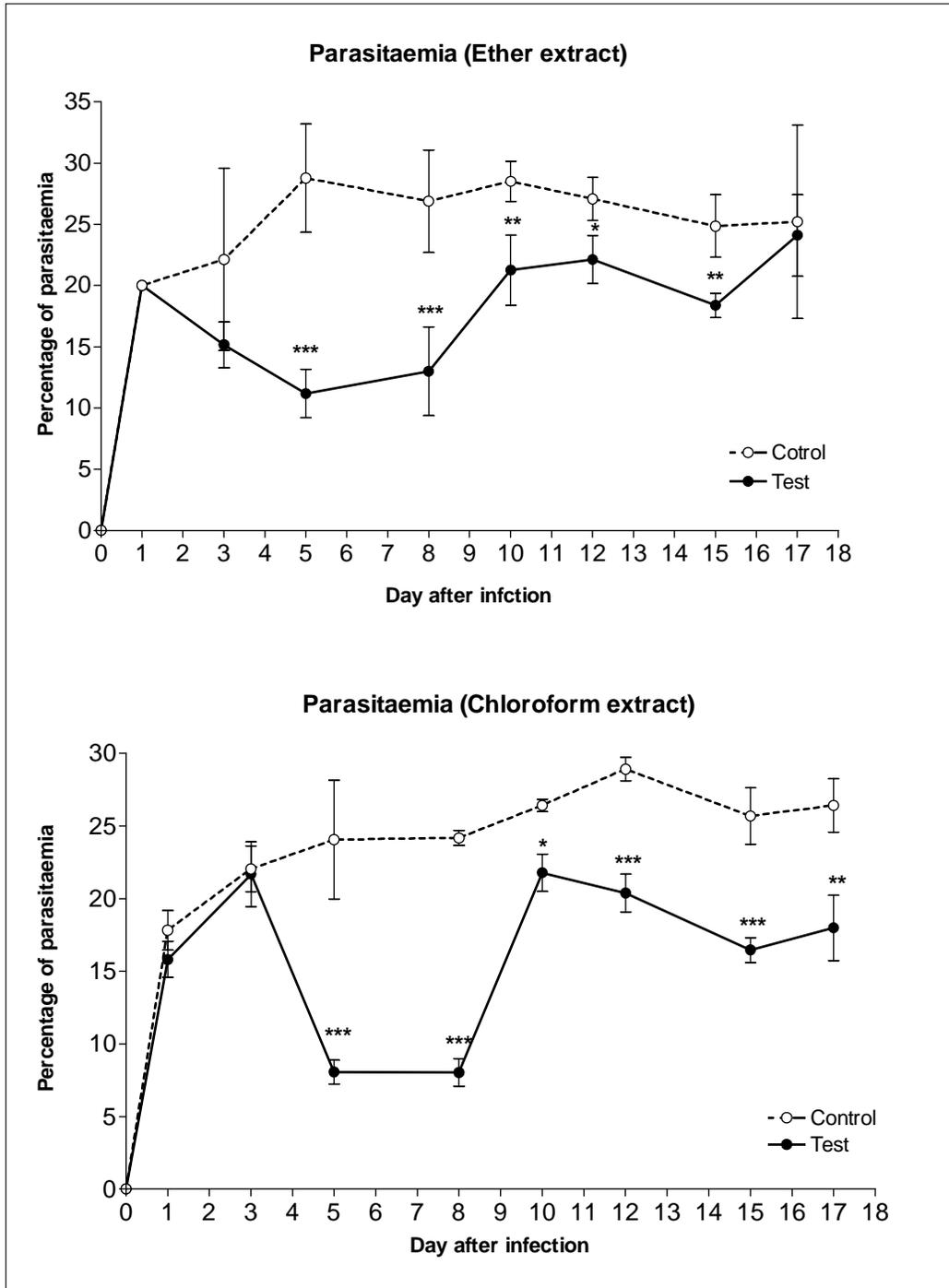


Figure 3: Comparison of the effects of *A. turanica* ether and chloroform extracts on parasitaemia in malaria mice Control (drug vehicle); Test (ether and chloroform extracts of *A. turanica*; n=5 mice/group/day, Statistical analysis using Student's *t*-test, * P<0.01, *** P<0.001).

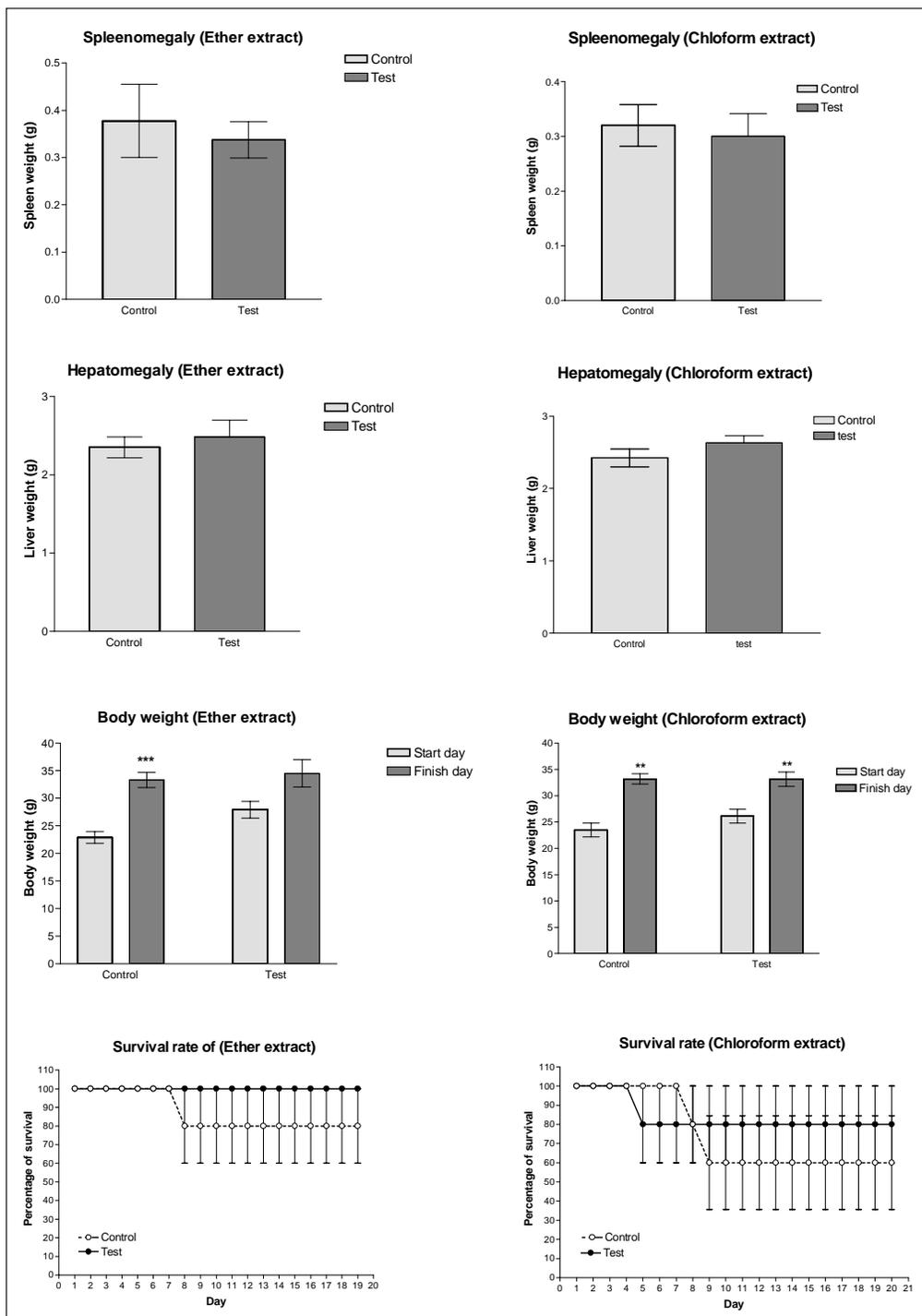


Figure 4: Pathophysiological evaluation after injection of *A. turanica* ether and chloroform extracts Control (drug vehicle); Test (ether and chloroform extracts of *A. turanica*; n=5 mice/group/day, Statistical analysis using Student's *t*-test, * P<0.01, *** P<0.001).

4. Discussion

Due to widespread antimalarial drug resistance, several plants have been used in traditional medicine for treatment of malaria and fever in many parts of world [47]. A number of antimalarial drugs have originated from plant sources and their potential productivity for novel agents has recently been emphasized [48,49]; the discovery of antimalarial property of artemisinin and its analogs is such an example [50,51]. Although, various species of *Artemisia* were used for their activity, only few species including *A. scoparia*, *A. sieberi* and *A. aucheri* are widely distributed in Iran [38]. This study is aiming on therapeutic application of *A. turanica* in malaria.

This study revealed no toxicity with even high dose of *A. turanica* crude extracts, which confirms its minimal side effects in naïve mice. In addition, ether and chloroform extracts were isolated from *A. turanica* and were successfully tested in *P. berghei* murine malaria. On the basis of authors previous publications [52,53], data of this study specifically indicated the inhibitory effects of the *A. turanica* extracts on the development of *P. berghei* by decreasing parasitaemia. The microscopic examination of Giemsa stained slides, showed a virtual absence of blood-stage of the murine malaria treated with these herbal extracts. These observations suggest that the active constituents in the extract may be cytotoxic for *P. berghei*, thereby inhibiting their development to the erythrocytic stage. Although, this study confirmed antimalarial effects of *A. turanica* extracts

in vivo, however there are more efficacies on pathophysiology by this medication. These observations may provide the basis for the traditional use of this herb in treatments of malaria disease [54].

The route of inoculation is important factor to determine herbal efficacy. Although, subcutaneous injection was used in this study, other routes may be recommended for future studies. Moreover, active derivatives of *Artemisia* including artemether, arteether and artesunate, are used by oral, intramuscular, rectal and intravenous administration [55]. In conclusion, the inhibitory effects of the *A. turanica* extract on the reduction of *P. berghei* parasitaemia, highlights its antimalarial activity, more investigations are required on different *Plasmodia* and hosts to clarify details of antimalarial effects of *A. turanica* and analysis of its natural components.

5. Acknowledgement

This work was funded by Department of Parasitology, Pasteur Institute of Iran and collaboration with Department of Applied Chemistry, Islamic Azad University, Qom Branch, Qom, Iran. This study has been involved a M. Sc. student thesis from Payame Noor University of Tehran centre, Iran under corresponding author supervision.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

Research funding: Pasteur Institute of Iran

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