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Research Article

Antimalarial Effects of Iranian Flora *Artemisia sieberi* on *Plasmodium berghei In Vivo* in Mice and Phytochemistry Analysis of Its Herbal Extracts

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The aim of this study is pharmacochemistry of Iranian flora *Artemisia sieberi* and its antimalarial effects on *Plasmodium berghei in vivo*. This is the first application of *A. sieberi* for treatment of murine malaria. *A. sieberi* were collected at flowering stage from the Khorassan and Semnan provinces of Iran; the aerial parts were air-dried at room temperature and then powdered. The powder was macerated in methanol, filtered with Bokhner hopper and solvent was separated in rotary evaporator. Total herbal extract was subsequently processed for ether and chloroform extracts preparation. The toxicity of herbal extract was assessed on naive NMRI mice with high, average and low doses; then pathophysiological signs were assessed. Finally, the antimalarial efficacy was investigated on two groups of *Plasmodium berghei* infected mice. Percentage of parasitaemia and pathophysiology were also evaluated. The results of this assessment showed no toxicity even by high concentration of herbal extract. A significant reduction in percentage of parasitaemia was observed; no alterations of hepatosplenomegaly and body weight were indicated in study group. *A. sieberi* extracts showed antimalarial effects against murine malaria with some efficacies on reducing pathophysiology. However, there is requirement to find the major component of this herbal extract by further studies.

1. Introduction

Malaria is one of the most serious and widespread diseases encountered by human. It is an infectious disease caused by the parasite *Plasmodia (P.)* transmitted by the female anopheles. Four identified species of this parasite exist, which cause different types of human malaria [1]. Although all the four species of malaria parasites can infect humans and cause illness, only *P. falciparum* is known to be potentially life threatening and some of infected persons die, usually because of delayed treatment [2]; however, annual incidence of clinically new cases and mortality rates are decreasing [3–6].

As malaria vaccines remain problematic, chemotherapy still is the most important weapon in the fight against the disease [7]. The antimalarial drugs including chloroquine, quinine, mefloquine, pyrimethamine, and artemisinin are currently used in malaria treatment. Part of the reason for the failure to control malaria is the spread of resistance to first-line antimalarial drugs, cross-resistance between the limited number of drug families available, and some multidrug resistance [8]. Resistance has emerged to all classes of antimalarial drugs except artemisinin, an endoperoxide antimalarial drug derived as the active component of *Artemisia annua*, a herbal remedy used in Chinese folk medicine for 2000 years "qinghaosu" [9–12]. Artemisinin is a natural product and a powerful antimalarial drug with significant activities, which has high potency whilst possessing low toxicity during treatment of malaria [13–15].

The genus *Artemisia* has always been of great pharmaceutical interest and is useful in traditional medicines for

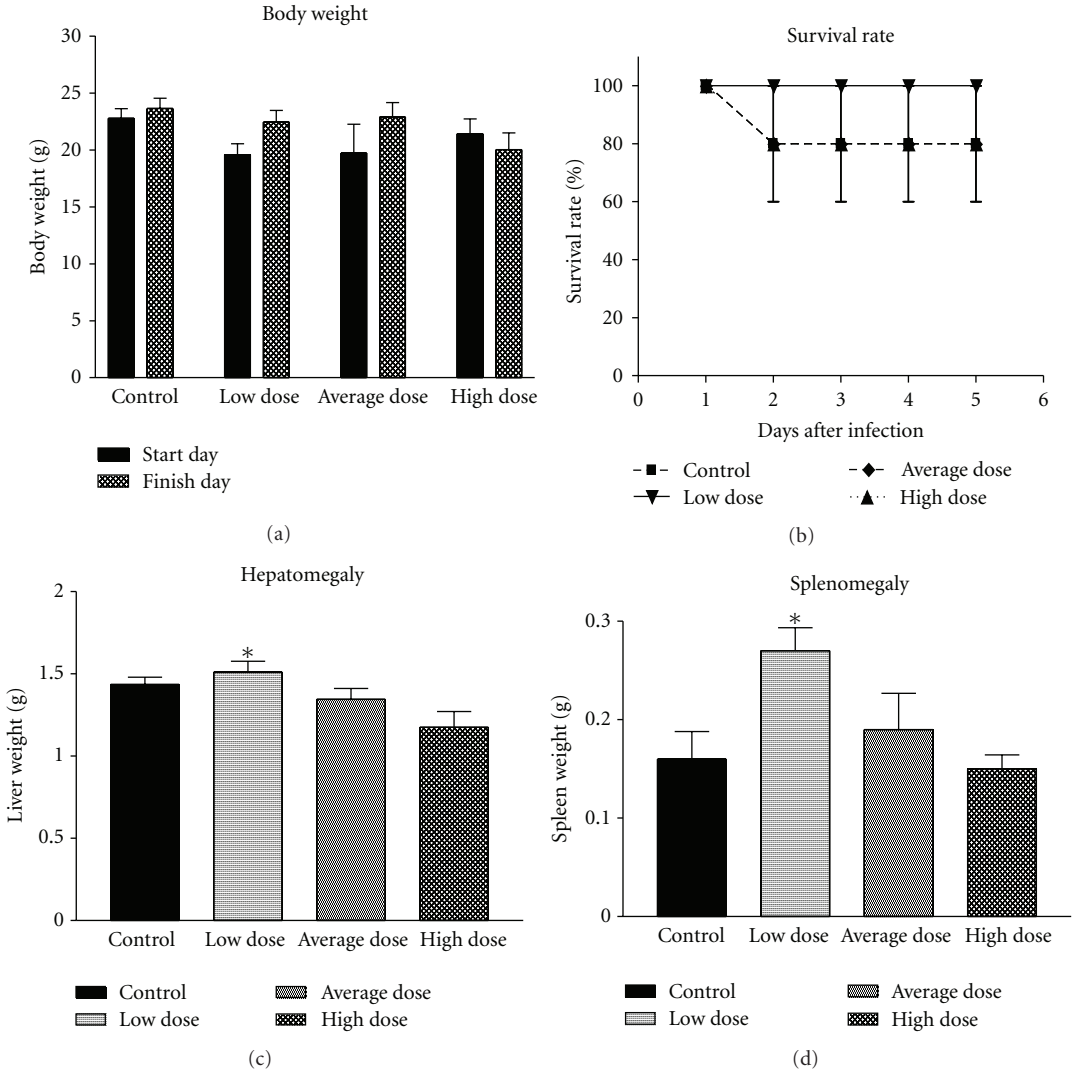

Figure 1: Toxicity assay induced by *A. sieberi* crude extract in naive animals. Pathophysiological alterations including body weight, survival rate, and hepato/splenomegaly were evaluated in control and test groups as toxicity assay induced by injection of low, average, and high doses of *A. sieberi* crude extract ($n = 5$ mice/group, Student's t-test, $^*P < 0.05$).

a treatment of the variety of diseases [11, 16, 17]. *A. annua* is presently being cultivated on a commercial scale in China and Vietnam for its antimalarial sesquiterpene lactone. The genus is of small herbs found in Northern temperate regions and belongs to the important family Compositae (Asteraceae), which comprises about 1,000 genera and over 20,000 species. Within this family, *Artemisia* is included into the family Anthemideae and comprises itself over 400 species, found in Europe and North America, but mainly are dominating Asia [18–20]. Among the Asian *Artemisia* flora, 150 species were recorded for China, 50 species reported in Japan, and 34 species found in Iran, of which may be endemic: *A. melanolepis Boiss* and *A. kermanensis Pold* [21], *A. absinthium* [22], *A. annua* [23], *A. dracunculus* [24], *A. aucheri* [25], *A. haussknechtii Boiss* [26], *A. scoparia, A. sieberi* [27], and *A. sieberi Besser* [28].

Pharmacochemical analysis of Artemisinin shows that the structure of this compound is rather unique among natural products as it contains the very unusual 1,2,4-trioxane ring system. It was sufficiently unusual that it was originally characterized as an ozonide until revised crystallographic analysis provided unambiguous structural elucidation [29–33]. For a drug to be effective against the malaria parasite, it must reach the site of action in sufficient concentration and then interact with the receptors before it is either deactivated and/or eliminated by the host or the parasite. Pharmacological and biochemical evaluation revealed that this compound was a blood schizonticide, preferentially imported into malaria infected erythrocytes via the parasitophorous duct [34] as it has been also shown in noninfectious diseases [35]. Due to complex chemical structure of artemisinin, the chemical synthesis of the molecule is complex, which results in very low yields, and the cost becomes prohibitory to use synthetic approach for its commercial production [36]. The mechanism of the action of Artemisinin remains a mystery; several candidates have been hypothesized as targets

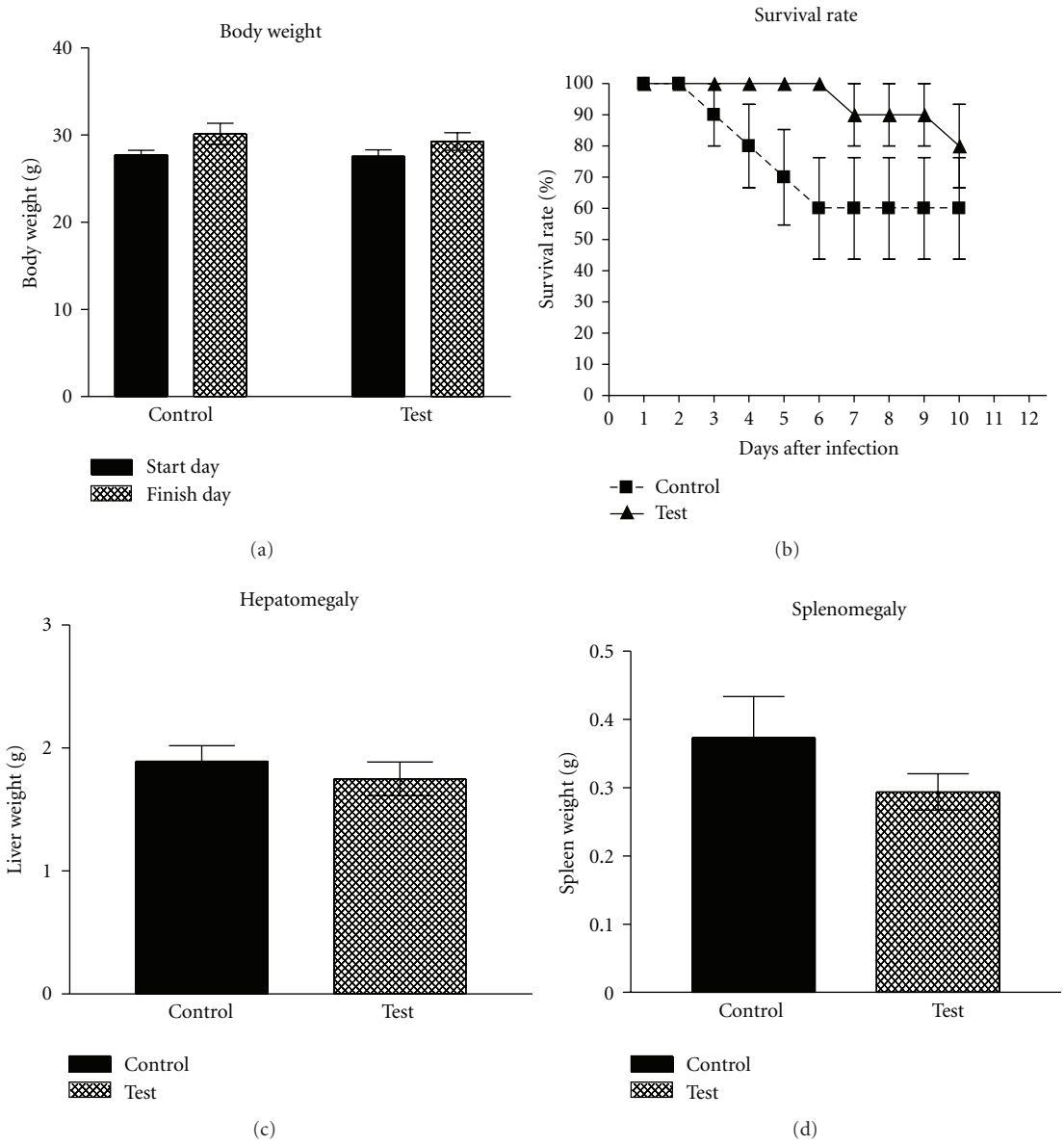

Figure 2: Toxicity assay induced by *A. sieberi* crude extract in malarial animals. Pathophysiological alterations including body weight, survival rate, and hepato/splenomegaly were evaluated as indices of toxicity by crude extract of *A. sieberi* in control and malarial groups (test, *A. sieberi* crude extract; control, drug vehicle; $n = 10$ mice/day/group, Student's t-test).

of artemisinin, including iron, haem, and some parasite membrane proteins [37–39], *Pf* ATP6 [40]; however, none of these has been convincingly shown to be functionally relevant and need to be debated [41].

Pharmacochemistry and chemical analysis of different genus of Iranian *Artemisia* species has been studied, and the presence of variety of components including monoterpenes [42], sesquiterpenes [43, 44], sesquiterpene lactones [45, 46], and essential oils [47–51] was fully reported [22–28]. The aim of this study is pharmacochemistry of natural components of Iranian flora *Artemisia sieberi* and its antimalarial effects on *Plasmodium berghei in vivo*. This is the first application of *A. sieberi* for treatment of murine malaria so far.

2. Materials and Methods

2.1. Plant Samples. The aerial parts of *A. sieberi* were collected at flowering stage from the Khorassan and Semnan provinces of Iran. Voucher specimens were deposited and identified at the Herbarium of the Research Institute of Forests and Rangelands (RIFR), Tehran, Iran.

2.2. Herbal Extraction. The method was applied as described previously [52]. The aerial parts were air dried at room temperature then were powdered by mixer. The powder (140 gr) of *A. sieberi* 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

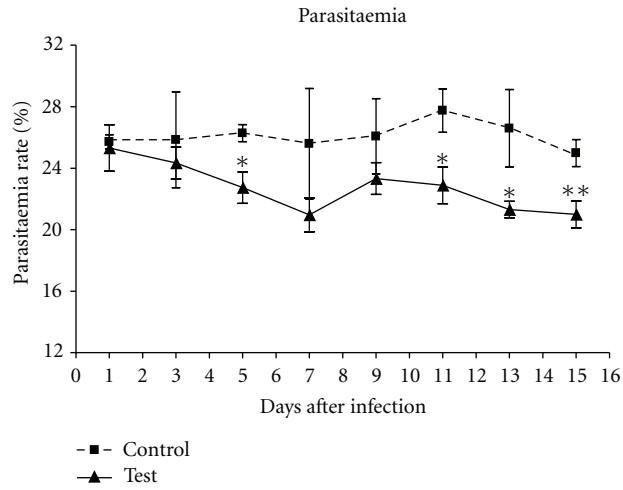

Figure 3: Percentage of parasitaemia in smears from blood of malarial mice. Smears were dried in air, fixed by methanol, and stained with Giemsa for counting of parasites inside red blood cells by light microscopy: test, *A. sieberi* crude extract; control, drug vehicle ($n = 10$ mice/day/group, Student's t-test, $^{*}P < 0.05$, $^{**}P < 0.01$).

(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.

2.3. Ether and Chloroform Extraction of A. sieberi 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 that 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 [52].

2.4. Animals. Male out bred 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 study. The mice were 4–6 weeks of age and almost 20 g weight which were housed at room temperature (20–23°C) on a 12 h light and 12 h dark cycle, with unlimited access to food and tap water.

2.5. Ethical Considerations. Experiments with animals were done according to the ethical standards formulated in the Declaration of Helsinki, and measures taken to protect animals from pain or discomfort. It has been approved by institutional ethical review board (Ethical Committee of the Pasteur Institute of Iran), in which the antimalarial test was done.

2.6. Experiments and Groups

(A) Toxicity Assay of A. sieberi Herbal Extract in Naïve Animals. In vivo toxicity was assessed by using herbal extract on naïve NMRI male mice. Animals were divided into four groups ($n = 5$ mice/group), including Group 1 (naïve), Group 2 (low dose), Group 3 (average dose), and Group 4 (high dose). According to several publications of this laboratory [51–54], in a blind experiment with no previous findings, three different concentrations ranging from 1 and 100 mg/mL can be used. A sample of herbal extract was suspended in ethanol and normal saline (1 : 9), then three different concentrations (low, average, and high doses) of herbal extracts including 1, 10, and 100 mg/mL were tested *in vivo* for their toxicity as test animals and a control group which was injected with drug vehicle. The parasite specificity of action was blood stage ring forms. Entire animals in all groups were injected with 200 μL of related solutions subcutaneously (sc) once a day for 5 days.

(B) Antimalarial Effects of Herbal Extract on P. berghei Infected Mice. Following toxicity assay, the highest dose with the lowest toxicity of herbal extract (100 mg/mL concentration) was selected to apply for its antimalarial activity on male NMRI mice infected with *P. berghei*. Animals were divided into two groups ($n = 10$ mice/group), including control and test; both groups were infected with murine malaria parasite, *P. berghei*. Drug vehicle and herbal extract were injected sc into control and test groups, respectively, once a day with 200 μL of solutions for the period of 10 days.

(C) Antimalarial Effects of Ether and Chloroform Extracts on P. berghei Infected Mice. The antimalarial efficacy of ether and chloroform extracts was investigated on murine malaria *P. berghei* infected NMRI mice. Animals were divided into four groups ($n = 5$ mice/group), including ether extract control and test, chloroform extract control and test groups. Drug vehicle and extracts were injected sc into control and test groups, respectively, once a day with 200 μL of solutions for the period of 14 days. Percentage of parasitaemia and pathophysiology were also evaluated.

2.7. Statistical Analysis. Values are presented as the mean ± SEM for groups of n samples. The significance of differences was determined by analysis of variances (ANOVA) and Student's t-test using Graph Pad Prism Software (Graph Pad, San Diego, CA, USA).

3. Results

Results of this experiment were classified in the following three steps including (A), (B), and (C).

(A) Toxicity Assay of A. sieberi Herbal Extract in Naïve Animals. No toxicity was observed *in vivo* even with high dose of *A. sieberi* total extract. Pathophysiological signs including body weight, survival rate, hepatomegaly, and splenomegaly represented no side effects of total extract (Figure 1).

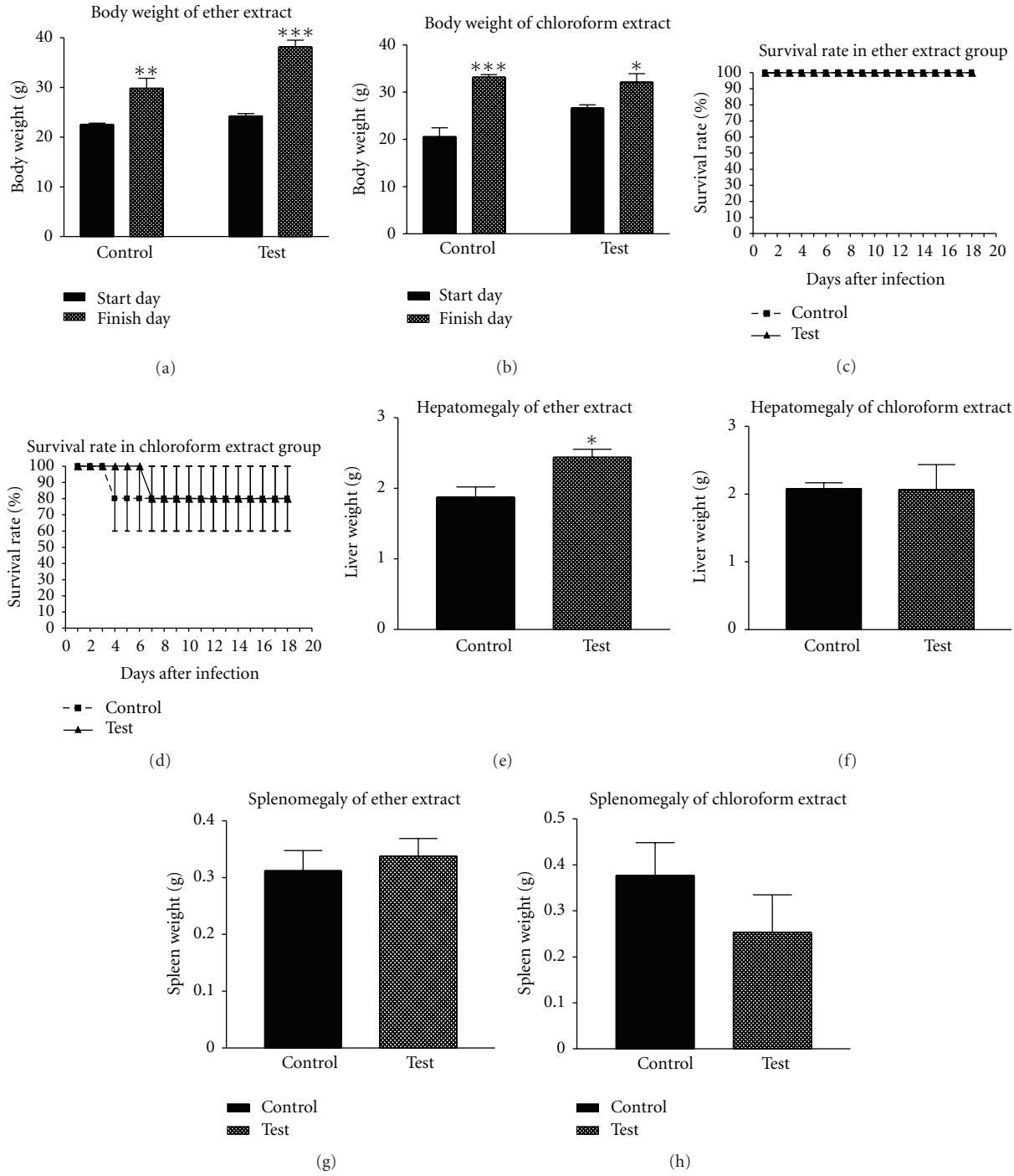

Figure 4: Pathophysiological alterations induced by *A. sieberi* ether and chloroform extracts in malarial animals. Pathophysiological alterations including body weight, survival rate, and hepato/splenomegaly were evaluated as indices of toxicity by ether and chloroform extracts of *A. sieberi* in control and malarial groups (test, *A. sieberi* ether and chloroform extracts; control, drug vehicle, $n = 10$ mice/day/group, Student's t-test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

(B) Antimalarial Effects of Total Herbal Extract on P. berghei Infected Mice. No side effects on pathophysiology were represented by total extract in malarial mice (Figure 2). The results indicated significant effects of total extract on reducing parasitaemia in test group in comparison with control group (Figure 3).

(C) Antimalarial Effects of Ether and Chloroform Extracts in P. berghei Infected Mice. Low pathophysiological changes including survival rate and hepatomegaly were indicated after treatment in test groups when compared with those in control groups (Figure 4). The results indicated the inhibitory effects of the *A. sieberi* ether and chloroform

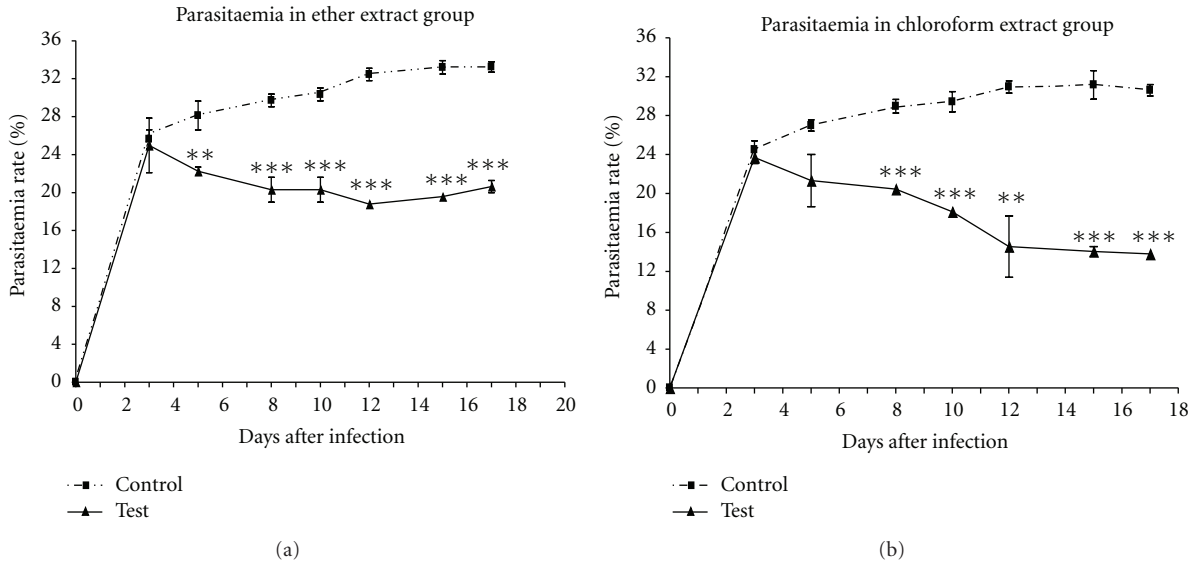

Figure 5: Percentage of parasitaemia induced by *A. sieberi* ether and chloroform extracts in malarial animals. Smears were dried in air, fixed by methanol, and stained with Giemsa for counting of parasites inside red blood cells by light microscopy (test, *A. sieberi* ether and chloroform extracts; control, drug vehicle, $n = 10$ mice/day/group, Student's t-test, $^{**}P < 0.01$, $^{***}P < 0.001$).

extracts on malaria by high reduction degree of parasitaemia (Figure 5).

4. Discussion

Although various species of the genus *Artemisia* were used for their pharmacological, antimicrobial, and antioxidant activity, only few species of this genus including *A. scoparia*, *A. sieberi*, and *A. aucheri* are widely distributed in desert area of Iran. This investigation is the first report on application of *A. sieberi* extracts on the treatment of murine malaria.

The results of this assessment showed no toxicity even with high concentration of herbal extract, which confirms its minimal side effects. In spite of less efficacy of crude extract of herb, ether and chloroform extracts were isolated from *A. sieberi* and were successfully tested in *P. berghei* murine malaria. Although a significant reduction was observed in the percentage of parasitaemia, no pathophysiological alterations were indicated in host hepato/splenomegaly and in body weight. The microscopic examination of Giemsa-stained slides showed a virtual absence of blood stage of the murine malaria treated with this 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.

In authors' previous publications [52–56], antimalarial effects of different Iranian flora of Artemisia herbal extracts including *A. turanica A. khorassanica, A. diffusa, A. absinthium*, and their effective agent (Tehranolide) against *malaria* and/or leishmania were successfully evaluated. 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. In addition to authors' previous publications [52–56], data of this study specifically indicated the inhibitory effects of the *A. sieberi* ether and chloroform extracts on the developmental stages 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. sieberi* extracts against murine malaria *in vivo* during infection; however, there are more efficacies on pathophysiological symptoms by this medication. These observations provide the basis for the traditional use of this herb in treatments of malaria disease.

Conclusively, the *A. sieberi* extract had antimalarial effects against murine malaria *in vivo*. Moreover, some efficacies are indicated on reducing pathophysiological symptoms by this medication. However, there is requirement to find the major component of this herbal extract by further studies. More investigations are required on different *Plasmodia* and animal hosts to clarify details of antimalarial effects of *A. sieberi* and analysis of its natural components.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

H. Nahrevanian and M. Kazemi conceptualized and designed the study. B. S. Milan conducted the daily monitoring of malaria parasitaemia and laboratory investigations. S. S. Mashhadi involved other laboratory assays and animal working. S. Nahrevanian and R. Hajhosseini involved in statistical analysis, preparation, and proofreading of the paper.

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