Original Article

Treatment of Cutaneous Leishmaniasis in Murine Model by Hydro Alcoholic Essence of *Artemisia sieberi*

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Abstract

Background: Considering the prevalence of leishmaniasis in Iran and many side effects associated with pentavalent antimony compounds use in its treatment, this study was designed to evaluate the effect of *Artemisia sieberi* essence on the experimental ulcers of cutaneous leishmaniasis on BALB/c mice.

Methods: This experimental research was performed to determine the effect of various concentrations of *Artemisia* essence in BALB/c mice previously infected with active *Leishmania major* promastigote. A total of 50 infected BALB/c mice were randomly divided into 5 groups. Three groups (30 mice) were used in the experimental conditions and the others were assigned as the control groups. The experimental groups received 1%, 3% and 5% of *Artemisia*, respectively. One of the control groups received ethanol 80% and the other received no treatment. The drug was administered by dropping the liquid on the top lesions, three times daily for maximum of 30 d. Every 10 days the ulcers diameter were measured and sampled for amastigote in all groups. Ulcers diameter changes were determined by statistical tests.

Results: After 30 days, diameter of CL lesions increased in 1%, 3% and 5% *Artemisia* concentrations and the control groups. Ulcers got bigger with the more concentration. Treatments could not reduce the diameter or caused small lesions. In addition, the mice direct smears in microscopic studies were positive.

Conclusion: To find the effective concentration and the mechanism of the effectiveness of the drug, further investigations with less concentrates of *A. sieberi* essence are recommended.

Keywords: Cutaneous leishmaniasis, *Leishmania major*, *Artemisia sieberi*, BALB/c

Introduction

Cutaneous Leishmaniasis (CL) represents a common health problem and standard treatments are often ineffective or yield poor cosmetic results (Berman 2003). CL is caused by *Leishmania* species belonging to the *Leishmania tropica* complex. Leishmaniases are transmitted by sand flies belonging to the genus *Phlebotomus* (Service 1996, Hepburn 2001, Markell and Voge 2006). It is found in most tropical and subtropical countries, but 90% of the estimated 1.5 million new cases each year occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria, where it is often associated with poverty (Hepburn 2001).

This ailment affects around 12 million people in 88 countries (WHO 1990). It is estimated that there are about 2-3 million new cases each year. It is also considered that presently there is a population of 350 million of people facing the risk of infection (WHO 1990, Iwu et al. 1994). Cutaneous leishmaniasis incidence average is 0.28 in each 1000 people

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in Iran. About 20000 CL cases are reported from different parts of Iran annually and estimated the rural number of cases exceeds this statistics (Mohebali 1996).

The classic treatment is pentavalent antimonials. The disadvantages of the antimonials are their requirement for intramuscular or intravenous injection each day for 20-28 days, their toxicity, and the recent development of resistance in same regions such as India (Berman 2003). Traditional treatment of CL is a common habit of natives in many endemic areas including many parts of Iran (Weigel et al. 2003).

Natural extract of different plants such as *Euphorbia* spp., *Gossypium herbacium*, and *Berberis vulgaris* are directly used on skin lesions as well as on the parasite in NNN medium (Fata et al. 2006). Artemisinin is an aromatic herb found in the extract of some medicinal plants such as *A. sieberi* (Tyler et al. 1988, Farzaneh et al. 2006, Baldi and Dixit 2007). This plant is an endemic species of Iran, which grows in many regions.

In order to evaluate the effect of *A. sieberi* essence on the experimental ulcers of CL on BALB/c mice, this study was undertaken over a 12-month period.

Material and Methods

This experimental study was done in Kashan University of Medical Sciences in 2006. This study was considered ethically approved by ethic committee of deputy of research of Kashan University of Medical Sciences. Seventy small laboratory white mice were inoculated subcutaneously by 0.1 ml liquid phase culture containing at least $5x\ l0^6$ promastigotes of standard *L. major* (MHOM/64/IR/ER75) (obtained from Tehran University of Medical Sciences). After 4 weeks, nodules and ulcers appeared on 50 inoculated mice. Fifty BALB/c mice were divided into 5 groups. Three groups (30 mice) were used in the experimental conditions (group A) and the others as control (group B).

The stems and leaves of *A. sieberi*, obtained from Kashan City, central Iran, were prepared, washed, dried, and extracted by Soxhlet Apparatus and solved in ethanol 80% in the Barij Essence Pharmaceutical Company.

One, 3 and 5 percent concentrations of hydro alcoholic essence of *A. sieberi* were used for CL lesions treatment. Before using essence, diameters of each lesion were measured. Different concentrations of the essence of *A. sieberi* were dropped on CL lesions of three groups of mice, three times a day, for 30 d. The placebo group (control group A) received ethanol 80% which was the solvent of the essence and the other group received no treatment (control group B).

The increase or decrease of diameters of each lesion was measured by metric caliber. At the end of each 10 d treatment period the ulcers diameter were measured and sampled for amastigote. Direct stained smear by Giemsa was prepared from the lesions of experiment and control groups at days 10^{th}, 20^{th} and 30^{th} after treatment. After the end of the treatment period, mice in the experimental and control groups were followed for 1 month.

The results were analyzed by Mean± SEM (Standard Error of Mean). ANOVA and LSD tests were used to examine the changes. A probability level of $P< 0.05$ was statistically considered significant.

Results

The results showed that after the end of treatment period, diameter of CL each lesion increased in 1, 3 and 5 percent of *Artemisia* essence concentrations and the control groups. Statistically significant increase in the size of ulcers observed in 3% and 5% concentrations and in the control groups ($P< 0.05$).

Treatments could not reduce the diameter or caused small lesions to disappear completely. These changes are shown in Table 1 and Fig. 1.

The examinations showed that using more concentrations of the essence caused more increase in diameter of CL lesions ($P< 0.001$). Direct Giemsa stained smears prepared from the lesions of experiment mice were positive for Leishman bodies and parasites were isolated from skin lesions. Ten days after treatments, secondary infections of the lesions in treated mice were seen and **developed** until the end of treatment period.

The figure shows that although the concentrations of 1%, 3% and 5% of plant drug could not reduce the size of the lesions or reduce the number of parasites, the increase diameter ulcers was less in treatments compared with control groups. Further analysis was performed comparing the amount of diameter lesions decrease in each treatment with control groups. The results of analysis showed that in 1% concentration group compared to CA and CB control groups, the size of ulcers was decreased 7 and 8.9 times, respectively. In the 3% concentration group comparing to CA and CB groups, the decrease of the diameter lesions was seen 1.2 and 1.6 times, respectively. In 5% concentration group comparing to CA and CB groups the decrease was seen 1.5 and 1.9 times, in that order.

One month after the treatment period, the sizes of ulcers in remained mice increased in the experimental and control groups and the mice direct smears in microscopic studies were positive.

Table 1. Average diameter increased of CL ulcers by different concentrations of *Artemisia sieberi*

Groups \ Diameter	Before treatment (mm)	After treatment (mm)	Increase (%)
1%	9.72	10.29	5.9
3%	10.67	14.2	33.1
5%	8.79	11.28	28.3
CA	7.53	10.63	41.2
CB	7.59	11.57	52.4

Control groups: CA= receiving ethanol 80% (placebo group), CB= received no treatment.

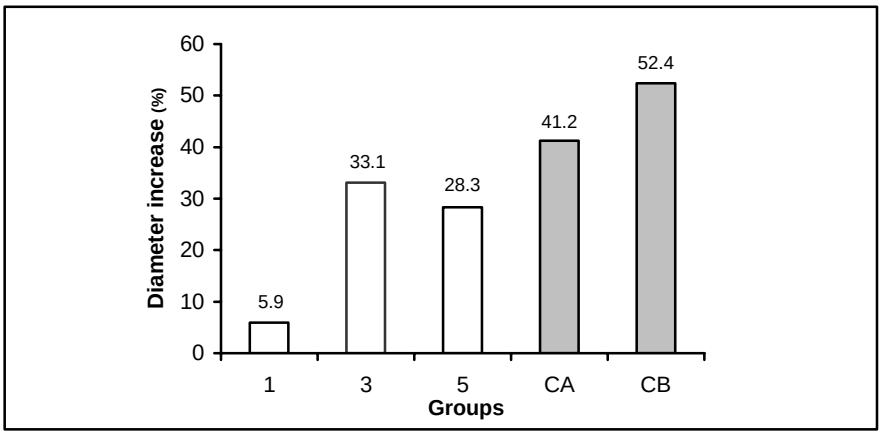

Fig. 1. Average diameter increased of cutaneous leishmaniasis ulcers of mice inoculated by *Leishmania major* treated by different concentrations of *Artemisia sieberi* compared to control groups: CA= receiving ethanol 80% (placebo group), CB= receiving no treatment.

Discussion

Artemisinin is a sesquiterpene lactone presents in a number of clinically important medicinal plants, including *A. annua* (sweet wormwood) and *A. sieberi* (Arab et al. 2006, Baldi and Dixit 2007). Artemisinin containing plants are used medicinally in virtually all traditional medical systems, and have a history of usage in Chinese medicine dating back to thousands years. Artemisinin has demonstrated significant antimalaria activity against *Plasmodium falciparum*, and *P. vivax* (Rita Bilia et al. 2002). Although widely used throughout the world as an anti-malarial, new studies show that this herb becomes cytotoxic in the presence of ferrous iron, and iron influx is naturally high in cancer cells. Case reports show effectiveness against a wide variety of cancers (Rowen 2002, Lai et al. 2005). On the other hand, studies showed some *Artemisia* species have antiviral and antiparasitic activity such as essential oil of the *A. arborescens* against HSV-1 and HSV-2 and active principle santonin of *A. nilagirica* against *Trichinella spiralis* larvae (Sukul et al. 2005, Saddi et al. 2007). Due to *Artemisia* species effects in pathogenic agents, the extract of *A. sieberi* effect in the *Leishmania* parasites in murine model was examined in this paper. However, we used the leaves of *A. sieberi* since is known that, it contained artemisinin (Arab et al. 2006). On the other hand, this plant is a common table vegetable all over Iran and abundant in Kashan as well as desert areas and access to this plant is very inexpensive, therefore essence of *A. sieberi* was used.

There is evidence that show aqueous extract and essential oil of *A. herba-alba Asso* has the strongest leishmanicidal activity in vitro. In one study, Aqueous extract and essential oil of Artemisia herba-alba Asso were tested for their antileshmanial activity again Leishmania tropica and Leishmania major. The strongest leishmanicidal activity was observed with the essential oil at 2 µg/ml as versus the other two strains tested. The aqueous extract showed an antileshmanial activity at 4 µg/ml (Hatimi et al. 2000).

In another study, methanolic demonstrated that extract of *A. aucheri* inhibited the *L. major* parasite multiplication at doses of 150, 300 and 450 µg/ml at 48 and 72 h of culture. Doses of 600 and 750 µg/ml showed the same effect at 24, 48 and 72 h of culture ($P< 0.05$) (Sharif et al. 2006).

The results of the studies mentioned above are not in agreement with the results of this study. This difference of finding may be due to the nature of studies conducted before. While the previous researches mentioned in this paper used in vitro approach, this study used in vivo method to examine the effectiveness of this treatment. However, it was concluded that *A. sieberi* essences were ineffective on *L. major* ulcers of BALB/c mice.

The present results shows, even though 1, 3 and 5 percent of concentrations of plant drug could not reduce the size of the lesions or cause small lesions to disappear completely and reduce the number of parasites, it could reduce the size of ulcers in treatment compared with the control groups.

The result of therapeutic effect of *Artemisia herba-alba* extract against cutaneous leishmaniasis by *L. major* in small white mice (out-bred) was negative and herb drug could not reduce the diameter of ulcers or the number of parasites (Babaee Khou et al. 2007). The size of ulcers was increased in all cases. This result is similar with that of our study.

There is several reason indicated lack of affect of *A. sieberi* essences on *L. major* and increase in the size of ulcers with the more concentration. They include ineffective major of active components of *A. sieberi*, essences and the other ingredients, genetic of reservoir host and effective genes on cellular immunity reactions, and genetic of species agent of the disease. Meanwhile, the number of parasite inoculated and the lesions secon-

dary fungi and bacterial infections development are also effective in treatment process. Thus, probably cytotoxic components in the plant drug are effective.

The major components of *A. sieberi* are camphor (54.7%), camphene (11.8%), 1, 8-cineol (9.9%), b-thujone (5.7%), a-pinene (2.5%) (Ghasemi et al. 2007). In another study on *A. herba-alba* (from Kashan area), comphor (42.5%), 1, 8-cineol (18.3%) and camphenes (8.7%) were recognized (Bamoniri 2004).

In conclusion, to find the effective concentration and the mechanism of the effectiveness of the drug, further investigations with less concentrates of *A. sieberi* essence are recommended.

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