

Treatment of Cutaneous Leishmaniasis in Murine Model by Alcoholic Extract of *Berberis vulgaris*

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Abstract

In order to evaluate the effect of *Berberis vulgaris* extract on the experimental ulcers of cutaneous leishmaniasis (CL) on Balb/c mice, a study was undertaken over a 12 months period. Forty Balb/c mice were divided into 2 main groups A and B. Each main group in turn was divided into 5 sub groups of 4 mice and each sub group were inoculated subcutaneously by 0.1ml liquid phase culture containing promastigotes of *Leishmania major*. After 2-3 weeks, nodules and ulcers appeared on 37 of 40 inoculated mice. Ethanol extract of the stem and leaves as well as roots of *Berberis vulgaris* in different concentrations, were used topically on CL lesions of 4 sub groups A and B, respectively. Ethanol alone was used on the lesions of control mice. The surface area of lesions were measured before and 1-2 weeks after treatment. Direct Geimsa stained smear prepared 20 days after treatment. The results showed that after 2 weeks, a statistically significant decrease of ulcer size of treated mice observed, while in the control group the lesion growth continued. The examinations showed that using higher concentration of the extract caused more decrease in surface area of CL lesions on day 15 and negative direct smear on day 20. Alcoholic extract of *B. vulgaris* root was more effective than leaves and stem extract. Alcoholic extract of *B vulgaris* might be further used in animal model.

Keywords: Cutaneous leishmaniasis, Treatment, *Berberis vulgaris*, Murine model

Introduction

Cutaneous leishmaniasis (CL) represents a common health problem and standard treatments are often ineffective or yield poor cosmetic results. The classic treatment is with pentavalent antimonials. The disadvantages of the antimonials are their requirement for intramuscular or intravenous injection each day for 20-28 d, their toxicity, and the recent development of resistance in same regions such as India (1).

Traditional treatment of CL is a common habit of natives in many endemic areas including Khorasan Province of Iran (2-3). 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 (4). Berberine is an alkaloid found in the extract of some medicinal plants such as *B. vulgaris* (5). In order to evaluate the effect of *B. vulgaris* extract on the experimental ulcers of CL on Balb/c mice, a study was undertaken over a 12-month period in the Dept. of Parasitology, Emam Reza Medical School, Mashhad, Iran.

Materials and Methods

Forty Balb/c mice divided into 2 main groups A and B. Each main group in turn was divided into 5 sub groups of 4 mice and each sub group were inoculated subcutaneously by 0.1ml liquid phase culture

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containing at least 5×10^6 promastigotes of *Leishmania major* (an isolate from Sarakhs district, Northeastern part of Khorasan Province, Iran). After 2-3 weeks, nodules and ulcers appeared on 37 inoculated mice. The rest 3 uninfected mice excluded from the study. The stems, leaves, and roots of *B. vulgaris* (an isolate from Birjand City) were prepared, washed, dried, and extracted separately by Soxhlet Apparatus in the School of Pharmacy. Different concentrations (2.5, 4.0, 5.5 and 7.0 percent) of the alcoholic extract of stems and leaves, in Vaseline base, rubbed topically on CL lesions of 4 sub groups A of mice two time a day for one week and ethanol alone was used on the lesions of control mice (fifth group). The same procedure was performed by similar concentrations of ethanol extract of *B. vulgaris* roots on the lesions of mice, sub groups B. The greater and lesser diameters of each lesion were measured by metric caliber and the surface areas of the lesions were calculated on days 1, 8 and 15 after treatment. A direct stained smear by Geimsa was prepared from the clinically healed and non-healed lesions of experiment and control groups. All results were expressed by mean \pm SEM (Standard Error of Mean). The significance

of difference was evaluated with ANOVA and LSD test. A probability level of $P < 0.05$ was considered statistically significant.

Results

The results showed that the surface area of CL lesions increased after one week in both treatment and control groups, but after 2 weeks, a statistically significant decrease of the lesion size of treated mice observed (Fig. 1 & 2), while in the control group the lesion growth continued ($P < 0.05$). The examinations showed that using higher concentration of the extract caused more decrease in surface area of CL lesions ($P < 0.001$). Comparing the extract of stem and leaves with extract of roots, the latter was more efficient especially in higher concentrations ($P < 0.01$). Most of the mice in control group died due to systemic leishmaniasis while all mice in treatment group were alive during the study period. Direct Geimsa stained smears prepared from the healed lesions of experiment mice were negative for Leishman bodies while in control mice (live or dead), parasites were isolated from skin lesions and visceral organs (liver & spleen).

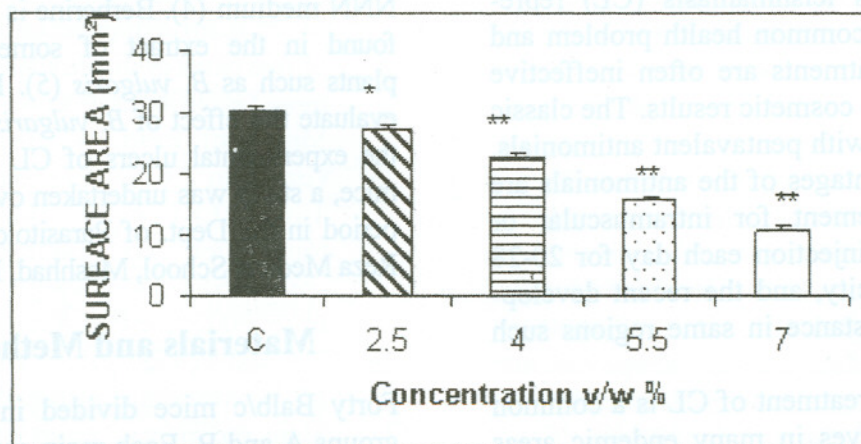


Fig. 1: Average surface area of CL ulcers of mice inoculated by *L. major* treated by different concentrations of alcoholic extract of *B. vulgaris* stems and leaves * $P < 0.05$ compared to Control, ** $P < 0.001$ compared to control

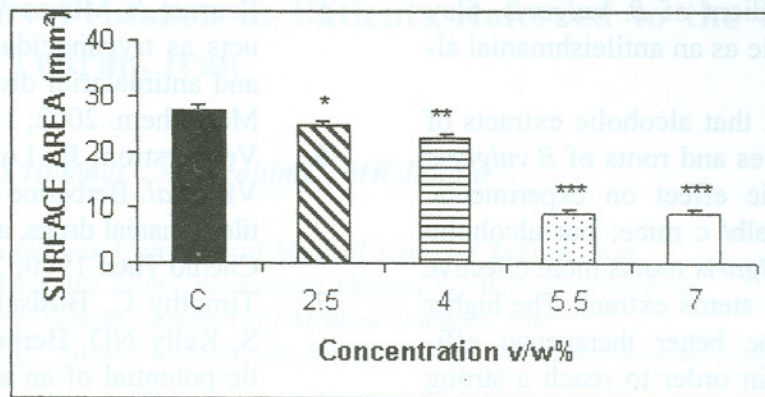


Fig. 2: Average surface area of CL lesions of mice inoculated by *L. major* treated by different concentrations of *B. vulgaris* root

* $P < 0.05$ compared to Control, ** $P < 0.01$ compared to Control, *** $P < 0.001$ compared to control

Discussion

Berberine is an alkaloid present in a number of clinically important medicinal plants, including *Hydrastis canadensis* (golden-seal), *Coptis chinensis* (coptis or golden thread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric) (5).

Berberine containing plants are used medicinally in virtually all traditional medical systems, and have a history of usage in Ayurvedic and Chinese medicine dating back at least 3,000 years (6). Berberine has demonstrated significant antimicrobial activity against bacteria, fungi, protozoa, viruses, helminthes and *Chlamydia* (7). Berberine also has other immunostimulatory effects. Sabir and Bhide have reported berberine stimulates blood flow to the spleen (8). Berberine has been shown to activate macrophages (9). Berberine sulphate has been used intralesionally in the treatment of human CL in Jodhpur, India (10). In another study, berberine sulphate was inoculated intralesionally on four occasions at weekly interval and was found to be highly effective against CL in domestic dogs (11). In another study, berberine was selected as a model drug to design a

transdermal delivery system for the treatment of CL (12). Not only berberine itself, but also its derivatives have been used as anti leishmanials (6).

In this paper, we used the leaves', stems', and roots' extract of *B. vulgaris*; as it was proved, they contained berberine (5). Honestly, it was difficult for us to isolate pure berberine from the plant. On the other hand, the natives in Khorasan Province use pure raw juice of *Berberis* tree on their CL lesions. Therefore, alcoholic extract of stem, leaf, and root was almost similar to the pure juice used in traditional treatment. Using different concentrations of alcoholic extract showed different results (Fig. 1 & 2). The extracts prepared from roots were more effective than stems and leaves that meant berberine concentration in root was more than in stem and leaf of *B. vulgaris*.

In this study vaseline was used as a base for the extracts, however, chitosan hydrogel has been used as a base for transdermal delivery of berberine in rat skin (10). In another study, successful treatment of oriental sore was reported by using intralesional berberine sulphate (8). Considering previous studies and the present one, it can be said that berberine as

an active ingredient of *B. vulgaris*, play the principal role as an antileishmanial alkaloid.

It is concluded that alcoholic extracts of both stems-leaves and roots of *B. vulgaris* have therapeutic effect on experimental CL ulcers of Balb/ c mice; but alcoholic extract of *B. vulgaris* root is more effective than leaves and stems extract. The higher concentration the better therapeutic efficacy, however in order to reach a strong conclusion, it might be further used in animal model.

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