

The efficacy of *Achilles millefolium* topical gel along with intralesional injection of glucantime in the treatment of acute cutaneous leishmaniasis major

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Abstract

Background: Leishmaniasis is still one of the endemic parasitic infections in many countries comprising Iran. During the past decades, several medical and surgical approaches have been applied and studied to achieve the best option to treat the cutaneous leishmaniasis in Iran and the world. This study was carried out to evaluate the effect of topical *Achilles millefolium* in conjunction with intralesional glucantime on acute cutaneous leishmanial lesions.

Materials and Methods: sixty patients with confirmed acute cutaneous leishmaniasis were recruited in the study. Patients were randomly allocated into two groups to receive twice daily topical gel of *Achilles millefolium* 5% (containing 5% poly phenol) (group A) or placebo (group B) for four weeks along with weekly injection of intralesional Glucantime.

Results: There was no significant difference between the two groups according to age, gender, and duration of the disease. Also, there was no significant difference in complete and relative cure rates between the two groups ($P = 0.35$) using Visual Analog Scale (VAS). Application site reactions were occurred in 12 patients including redness in 8 cases in group-A and 2 cases in group-B, severe itching in one case in group-A and increasing wound secretion in another case in group-A ($P = 0.014$).

Conclusions: Given the result of the present study, there is no significant difference in cure rates of lesions between yarrow and placebo topical gels as an adjuvant drugs with intralesional glucantime in treatment of acute cutaneous leishmanial lesions.

Key Words: Cutaneous leishmaniasis, *Achilles millefolium*, glucantime

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INTRODUCTION

Leishmaniasis is a widespread parasitic infection with a vast spectrum of manifestation from local cutaneous to visceral form. The most common manifestation of the cutaneous disease is localized form lesions. Cutaneous leishmaniasis has been seen in over 88 countries with an annual incidence of 1 to

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1.5 million cases.^[1] However, more than 90% of the cases are reported in just six countries comprising Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria.^[1] It is suggested that the reported an annual incidence of the disease is probably underestimated as leishmaniasis is a notifiable disease in only 40 of those 88 countries.^[2]

An infected female sand-fly by protozoa leishmaniasis could transmit disease by biting.^[3] The results of infection vary from a chronic skin ulcer to erosive mucosal disease and it depends on both infecting species of the protozoa and the immune response of the host.^[4]

Nowadays, the first-choice treatment for leishmanial infection is pentavalent antimonials, which are potentially toxic and often ineffective in most of the cases. The second-line compounds that generally used in treatment of the unresponsive cases include pentamidine and amphotericin B. However, as these compounds pose a high toxicity risk to human, a 30-year advanced search for non-parenteral and relatively non-toxic anti-leishmanial agents has been generated.^[5-7]

Achillea millefolium (Yarrow) gel is one of traditional herbs that were studied in the past for its wound healing effect. Later in 2005, an experimental study was carried out by Luize and colleagues^[8] that revealed *Achillea millefolium* has also sufficient effect on healing of the leishmanial lesions. Nevertheless, its exact mechanism in clinical models is not still clear. This is the first randomized clinical trial evaluating the efficacy of *Achillea millefolium* in cutaneous leishmaniasis.

The purpose of the present study was evaluation of efficacy of *Achillea millefolium* topical gel along with intralesional injection of glucantime in the treatment of acute cutaneous leishmaniasis lesions.

MATERIALS AND METHODS

Study population

This is a double-blind, randomized placebo-controlled study that conducted from January 2009 to February 2010 approved by Isfahan University of Medical Sciences Ethical Committee by the code of 387419. Patients with severe leishmaniasis who were referred to our university medical and research center were enrolled into the study. All patients gave written informed consent to participate, and the study protocol was approved by the regional ethics committee.

Eligible participants were of Isfahan province, met the histological criteria for presence of parasite, age of

older than 5 years, acute leishmanial lesions smaller than 5 cm², less than 5 lesions in each patient, more than 3 months of diseases duration.

Exclusion criteria comprised of pregnancy, women in fertility age, history of administration of immune suppressive drugs in the last six months or anti-leishmanial drugs in the last month. In all cases the diagnosis was based on positive smear and/or cultures. The largest lesion was selected for smear and culture.

Four study visits was scheduled for all of the patients; baseline (inclusion), month-1, month-2 and month-3 visits.

Drug and randomization

During this double-blind randomized study, 60 patients were randomized into two treatment groups by using random allocation computer software. All patients received weekly intralesional injection of Glucantime (Glucantime, Paris, France) at a dose of 20 mg/kg/day for 4 weeks and twice daily topical gel of 5% yarrow (containing 5% poly phenol) (group-A) or placebo (group-B). Therapy gels were prepared in two steps. Carbomer 934 was dispersed in distilled water and neutralized with triethanolamine to increase its continuity. Dried *Achillea millefolium* was dispersed in glycerin 20% and propylene glycol 5%. Parabens 2% and 100 g water were added to this solution to increase its solubility. Finally two solutions were mixed together and stirred to obtain a suitable dispersion. The placebo gel was prepared using the same material except for the plant extract and chlorophyll was used as coloring agent. Both gels were identical in terms of the color and consistency. The reactions included Papule, Ulcer, Plaque and Nodule were evaluated and registered. The lesions and their cure rate were evaluated by visual analog scale (VAS).

Statistics

Data were reported as mean \pm standard deviation (SD) when normally distributed, otherwise as median and range. The *t*-test and Fisher's exact test were used for the comparison of continuous and categorical data, respectively. Due to the small sample size and the distribution of data, the non-parametric Friedman Test for repeated measures was used to analyze changes over time within groups also Kaplan-Mayer tests were used. A *P* < 0.05 was defined as statistical significant. All analyses were carried out using SPSS for Windows version 16.0.

RESULTS

There were 60 patients in the study comprised of 45 (75%) male 15 (25%) female with mean age of

25.29 ± 4.01. Both groups were similar in age, sex, baseline and lesions characteristics and clinical findings ($P > 0.05$) shown in Table 1. In group-A, there were 8 patients with papular lesions, 16 patients with ulcerative lesion, 10 patients with plaque lesions and 10 patients with nodular lesions. In group B, 9 patients had papular lesions, 16 patients had ulcerative lesion, 11 patients had plaque lesions and 12 patients had nodular lesions.

After 6 weeks, 10 patients in group A and 12 patients in group B had positive smear sample but this difference wasn't statistically significant ($P > 0.05$).

There were no significant cure rates (complete and partial according to the VAS) differences between the two groups according to different follow-up times [Table 2]. Based on Kaplan-Mayer test, rate of the cure between the two groups wasn't statistically significant ($P = 0.35$).

At the end of follow-up time in group-A, eight patients had mild or moderate-severe itching and redness, one patient had severe itching and one patient suffered from increasing wound discharge. There were just 2 patients with mild itching in group-B ($P = 0.014$).

DISCUSSION

Cutaneous leishmaniasis lesions range from small nodules to gross mucosal tissue destructions. The mainstays of the treatment in cutaneous leishmaniasis are mostly pentavalent antimonials. As these agents

pose human to a high toxicity risk, the main problems of treatment with pentavalent antimonial drugs are serious albeit reversible side effects. These side effects include musculoskeletal pains, renal failure, hepatotoxicity, and cardiotoxicity.^[9,10] Moreover, long duration of the standard treatments (intramuscular or intravenous injections) results in low compliance of the patients to complete full course of the treatment.^[11] As a result, many studies have been performed to detect suitable alternative therapies for cutaneous leishmaniasis to reduce systemic toxic effects, economic cost, and poor treatment compliance of patients.^[10] Hence, several topical medications and traditional herbs began to be focused and applied in different parts of the world. Quinacrine, miconazole, clotrimazole, chlorpromazine, amphotericin, garlic cream and ZHE cream are some of these medications in treatment of cutaneous leishmaniasis lesions.^[12-17]

In the present study we used *Achillea millefolium* gel in combination with intralesional glucantime for treatment of acute cutaneous leishmaniasis lesions. The herein results demonstrated no superiority of *Achillea millefolium* gel along with intralesional injection of glucantime to placebo in the treatment of acute cutaneous leishmaniasis lesions.

However, it is not in line with Luize *et al.*^[8] findings that showed *Achillea millefolium* statistically inhibited growth of the promastigote and amastigote form of the leishmania amazonensis. They also signified that *Achillea millefolium* has no cytotoxic and hemolytic effects on sheep blood at concentrations of 100, 500 and 1000 µg/ml after either 60 or 120 minutes of incubation.

However, our present result is in some part corresponding to our previous study on evaluation of other extracts of *Achillea millefolium* in combination with intralesional glucantime.^[18] The study on Balb/c mice demonstrated that hydroalcoholic extracts of *Achillea millefolium* had higher efficacy than systemic glucantime or alcohol alone on treatment of cutaneous leishmaniasis lesions.^[18] Although, we could not prove the adjuvant therapeutic effect of other topical agents such as honey together with glucantime in treatment of cutaneous leishmaniasis.^[19] Actually, the study revealed that combination therapy of honey with intralesional glucantime reduces the effects of glucantime on healing process of leishmaniasis lesions.^[19] Also there are some other studies that have evaluated effect of some topical medications or traditional herbs alone in comparison with intralesional or systemic agents and demonstrated insufficient effect of these agents in treatment of cutaneous leishmaniasis.^[20,21] One of these randomized

Table 1: Baseline and lesions characteristics in two groups

Characteristic	Group (N=30)		P value
	A (Yarrow)	B (Placebo)	
Age (mean±SD)	25.8±3.7	24.6±4.2	0.250
Gender (%)			
Male	23 (76.6)	22 (73.3)	0.500
Female	7 (23.3)	8 (26.6)	
Number of lesions per patient	1.56±0.8	1.52±0.4	0.798
Type of lesions (%)			
Papule	8/44 (18.1)	9/38 (23.6)	0.990
Ulcer	16/44 (36.3)	16/38 (42.1)	
Plaque	10/44 (22.7)	11/38 (28.9)	
Nodule	10/44 (22.7)	12/38 (31.5)	
Duration of disease (Months)	3±0.5	2.7±0.7	0.104

Table 2: Comparison of complete or relative cure rates between the two groups according to the time of follow-up

Follow-up time	Group (N=30) (%)		P value
	A (Yarrow)	B (Placebo)	
Week-four	16 (53.3)	12 (40)	0.251
Week-eight	18 (60)	16 (53.3)	0.458
Week-twelve	21 (70)	18 (60)	0.351

clinical trial conducted on 96 patients with cutaneous leishmaniasis and showed topical paromomycin was less effective than intralesional meglumine antimoniate in the treatment of cutaneous leishmaniasis.^[21] Another study showed no significant efficacy for 2 to 5% hydroalcoholic extract of *Cassia fistula* had in the treatment of leishmaniasis lesions. However, higher concentrations (25 and 40%) resulted in significant reduction of cutaneous lesions in comparison with control group.^[20] According to such controversies regarding effectiveness of administration of topical agents alone or in combination with intralesional and systemic agents in cutaneous leishmaniasis, make us to plan more and better designed prospective studies to achieve the best results.

We also detected some side effects of and application site reactions to the *Achillea Millefolium* in the study. Among the patients who had been received Yarrow, redness, severe itching and increased wound secretions and a volcano like lesion were developed. The contact irritancy effects such as increased wound secretion, volcano like lesion of *Achillea Millefolium* have been previously demonstrated.^[22-24] It has been suggested that Alpha-peroxyachifolid and thujone components of *Achillea millefolium* may be responsible for contact dermatitis reactions.^[24] However, there is no general consensus regarding responsibility of *Achillea millefolium* for such sensitization response in topical administration.^[23]

In conclusion the present study showed that combination therapy of *Achillea millefolium* gel and intralesional glucantime has is not statistically effective than intralesional glucantime alone in the treatment of acute cutaneous leishmaniasis lesions.

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