Assessing the effectiveness of Aloe Vera 2% Gel in treatment of Cutaneous Leishmaniasis: Random clinical trial

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Abstract

Cutaneous leishmaniasis is a skin disease with long-lasting wounds. The current standard treatment methods have such problems as potential side effects, specific route of administration and drug resistance. This study was intended to examine the effect of Aloe Vera on wound healing in cutaneous leishmaniosis. This was a randomized clinical trial of 100 patients who were divided into two groups. The intervention group was administered with A. Vera gel 2%, and the control group was also given a placebo. Both groups also received standard treatment of cutaneous leishmaniasis. Before the onset of treatment and end of each week until the lesion showed complete epithelialization, the degree of healing and clinical characteristics of the wound (including wound size, skin induration size, and epithelialization rate) were measured and recorded. The results showed that the complete recovery in the intervention group was higher
than that in the placebo group \( (p= 0.126) \). In addition, the treatment results in the intervention group was better than that in the placebo group \( (p= 0.471) \). According to the results, the mean days of treatment was lower in the intervention group than the placebo group \( (p= 0.95) \). These differences were not statistically significant. A statistically significant difference was observed between the two groups in the mean diameter of the wound \( (p= 0.012) \). An A. Vera gel %2 in combination with a standard treatment protocol may improve wound healing and the recovery rate of cutaneous leishmaniasis lesions.

**Keywords:** Cutaneous Leishmaniasis, Aloin, Aloe Vera gel, Medicine, Traditional

**Introduction**

The World Health Organization (WHO) listed leishmaniosis as one of the six major parasitic diseases in tropical regions [1]. Despite significant advances in leishmaniosis treatment, the disease remains a major health problem in several countries [2]. This disease is present in all parts of the world and is endemic in 90 countries [3]. According to the WHO, over 350 million people are at risk of developing this disease [4]. The number of people with leishmaniosis is estimated to be about 12 million, and about 2 million new cases of leishmaniasis occur annually, with about half a million people diagnosed with visceral leishmaniosis (kala-azar) and one and a half a million people with dermal leishmaniosis (cutaneous leishmaniosis) [5]. Iran is one of the 7 countries where 90% of the world cutaneous leishmaniosis is reported [6]. About 20,000 new leishmaniasis cases are reported from Iran every year; however, the real number of cases is probably more than 4 to 5 times higher than this figure. The highest prevalence rate in Iran is found in Mashhad, Neyshabur, Shiraz, Kashan, Bam, Isfahan, Sarakhs and Golestan [3].

The cutaneous leishmaniosis is usually healed spontaneously within 4-12 months. However, it can result in egregious scars. Therefore, treatment shortens the duration of the disease and reduces scaring. The prevention of chronic lesions is a necessity in the early stages of treatment. In addition, treatment of cutaneous leishmaniasis may decrease the severity of the wound infection, have psychological impacts, and interrupt the cycle of transmission. In some cases, the wounds become larger and leave disfiguring scars. Current therapies for cutaneous leishmaniosis include limited medications such as pentavalent antimony compounds, amphotericin B, and
pentamidine [3-5]. Cutaneous leishmaniosis is mainly treated with glucantine in Iran’s healthcare system. Improper treatment of cutaneous leishmaniosis can lead to permanent disfiguring scars, which necessitates provision of healthcare services. However, the limited number of drugs [7], toxic and serious side effects of medications [3], development of drug resistance [8, 9], lack of positive response to treatment in patients infected with HIV, lack of a vaccine for the disease, drug cost and patients’ affordability warrant the need for developing innovative therapies.

On the other hand, considering the high capacity of traditional medicine in Iran and the general interest of the people in the non-medical treatments and the great emphasis of the World Health Organization and other organizations active in the field of health, on the use of traditional medicine capacities of the countries [10], we felt that, in line with the scientists in other countries, we should study the indigenous therapeutic and herbal remedies in our country along with existing chemical treatments. In this regard, it is necessary to carry out clinical trials to evaluate the therapeutic methods present in the traditional medicine texts and to update and utilize them in case of being effective.

Studies showed that leishmaniosis is one of the oldest diseases in human history and its records date back to 650 BC. Abu Ali Sina described the disease as “Balkh sore” in the 10th century. This disease was discussed in detail in the traditional Iranian textbooks and various methods and combinations were proposed for its treatment. One of the recommended methods is topical use of ointments containing alovine [11, 12]. Alovine or Aloe Vera is a plant exhibiting good results in wound healing and anti-leishmanial properties in the newest studies [13, 14].

In this study, the effectiveness of 2% A. Vera gel on the treatment of the cutaneous leishmaniosis was investigated in patients referring to the Gonbad treatment center in northeastern Iran in 2015 and it was compared with the control group.

**Materials and methods**

**Design of the study and ethics statements**

This study was a randomized placebo-controlled double-blind parallel-group clinical trial which accordance with the proposed methodology of the World Health Organization (WHO) for clinical trials for treatment interventions on the cutaneous leishmaniosis. For the study, the Medical Code of Ethics (Approval No. 1751- IR.MAZUMS.REC.94, 2015.09.30) was obtained
and it was registered with IRCT2015101224490N1 code in the Iranian Registry of Clinical Trials.

Sample size and study population

The study population included patients who referred to the health center of Gonbad-e-Kavos in the north-east of Iran during the period from October 1, 2015, to December 15, 2015, and the certain diagnosis of cutaneous leishmaniosis was indicated for them.

The patients referred due to skin lesions lasting for more than 14 days and with a clinical suspicion (since the diagnosis and treatment of the cutaneous leishmaniosis are performed by the health system and free of charge at the center of the cutaneous leishmaniosis, all patients with cutaneous leishmaniosis referred to this center). The lam was prepared from inflamed and swollen edges of patients’ lesions with a sterilized scalpel, and if the Leishmania parasite was found by the microscope after staining the cover glass with Giemsa, a definitive diagnosis of the cutaneous leishmaniosis was given. The patients were included in the study after the complete explanation of the research in case of being satisfied. Of course, based on the following criteria the patients were excluded from the study: the pregnant women, the patients with a history of drug allergy to antimony compounds, the patients with more than three months since the onset of their first skin lesions, the patients receiving any treatment for cutaneous leishmaniasis within a month and the patients with wounds on the eyelids and nose, or within less than 1 cm near the ends of the lips and eyes, as well as non-use of the drug, the reluctance to continue to participate in the study or to use another therapeutic approach. All patients were treated with standard cutaneous leishmaniosis treatment, including the topical or muscular injection of glucantine with or without cryotherapy.

The sample size was determined as 45 people for each group at the confidence level of 95% and test power of 80%. The patients were randomly assigned to two groups of intervention and control. The first group, received also a 2% A. Vera gel in addition to the standard treatment, and in order to prevent bias of the second group, a placebo also in the form of a gel was given to the second group, similar to the drug in terms of its shape, color and odor was, and in terms of the combination it did not just contain the active ingredient of A. Vera.

Drug and placebo preparation
To prepare the gel, the powder of the *A. Vera* extract was used. According to the contamination of the extract powder, the extract was sterilized by radiation method and by the Iranian Atomic Energy Organization. Then, 0.15 g of methyl paraben was dispersed in 20 g of glycerin. Then, 2 g of Aloe Vera extract was added to glycerin and 0.5 g sodium benzoate was added to sterile distilled water. Glycerin was mixed with distilled water and the mixture became completely homogeneous using a mixer. One gram of carbople was added to the formulation into the beaker and placed in a refrigerator for 24 h after closing the breaker opening with paraffin. After 24 hours, the formulation was removed from the refrigerator and triethanolamine was added to the beaker contents under a stirrer at 500 rpm to form a gel. After neutralizing the desired gel was obtained. The stability of the product was investigated at ambient and refrigerator temperatures, occurring opaqueness, synergistic phenomena, the apparent change in consistency, and pH. Microbial control of the product was evaluated and approved based on the American Pharmacopoeia (USP), after obtaining a suitable culture medium for microbial control, and the quality of the gel was examined by determining the total anthraquinone content.

The use of the gel was repeated two times a day at the intervals of 12 h, and its use continued until complete epithelialization of the lesion. The use of the drug and the placebo were also given to the patient in a sheet in addition to the oral explanation.

**Randomization and blinding**

The sample size was determined as 55 people for each group at the confidence level of 95% and the test power of 80%.

Random allocation software Ink (Version 1.0, May 2004) was used to create a randomization table by a block size of four. Therefore, the patients were allocated to A and B groups according to the randomization table, respectively. This study was a tow blind trial. The herbal extract and placebo were delivered to the patient in a similar laminate tube marked as “A” or “B”. A physician in leishmania center assessed the lesions of both groups, blindly. Moreover, the person who performed the statistical analysis was blinded and was only aware of the groups by the allocations “A” or “B”.

**Data collection**
At the beginning of treatment, a questionnaire was completed for all the patients, and their demographic data, morphological information of the wound (including a number of wounds, wound site, wound shape and duration of wound incidence), treatment information and clinical information of the wound were recorded.

Before the onset of the treatment and the end of each week (until complete wound epithelialization), the healing rate and clinical information of the wound (including the size of the wound, the size of the induration and the degree of epithelization) were calculated and recorded by the trained person not being aware of the treatment groups. The extent of wound healing was measured during and after the treatment, on this basis, the rate of clinical healing of the wound was determined as the following: in the case of the complete epithelialization, the lesion was completely healed, in the case of the reduction of more than 50% the lesion was moderate healed complete, and the reduction less than 50% of the lesion size was defined as slight healing. Lack of any apparent change in the clinical status of the lesion was defined as the non-response, and any increased lesion or deterioration of its clinical status was defined as worsening. If the lesion was not healed completely during the treatment course or 4 weeks after it, the outcome of the treatment was defined as improved, and if the lesion was still active, after 4 weeks of complete treatment, the outcome of the treatment was defined as the failure. The cases with recurrence or subsequent treatment failure after at least two complete systemic treatment courses were considered as clinical resistance.

**Interventions and follow up**

All the patients underwent the standard cutaneous leishmaniosis treatment, which included: local or intramuscular injection of glucantime with or without cryotherapy. The use of gel was repeated twice a day at the intervals of 12 hours and continued until the complete epithelialization of the lesion. The drug was to in a way that a first the wound area was washed with plumbing water or sanitary water and soap, then the lesion was disinfected with savlon, after drying. After drying the lesion it was covered with a thin layer of ointment slowly and the place was kept dry for about 2 hours. It was necessary to avoid taking the other medicine at the same time. The instruction of taking the medicine and placebo was delivered to the patient in a paper in addition to verbal explanation.
Meanwhile, a phone number was provided to the patient or the patient’s caregiver to report the probable complications during the day.

At the beginning of treatment, two forms of the questionnaire were completed for all the patients. The demographic data, morphological information of the wound (including the number of wounds, wound site, wound shape and duration of wound incidence), treatment information, and clinical information of the wound was recorded. Before the onset of treatment, at the end of the first, second, third, fourth, and eighth weeks of the treatment onset, the degree of healing and clinical information of the wound (including the size of the wound, the size of the induration and the degree of epithelization) were measured and recorded by the physician of the cutaneous leishmaniosis center, who was not aware of the treatment groups. The extent of the wound was measured during and after the treatment and the rate of wound healing was determined. Possible clinical complications were also recorded in the questionnaires. The patients who did not take full medicine or did not want to continue participation in the study were excluded.

**Statistical analysis**

To describe the qualitative variables from frequency distribution tables, and to determine the relationship between these variables, non-parametric Chi-square tests were used and Fisher's exact test was used, if necessary. In this situation, the Mantel-Hansel method was used to modify the confounding variables, and multiple logistics regression was used as needed. According to the repeated measurements of the qualitative dichotomous variable, Cochran test was used. Mann Whitney nonparametric test was used to measure the effect of the intervention on ranked qualitative variables or abnormal quantitative variables in both groups. Quantitative data were described using mean and standard deviation and normal distribution of quantitative variables were performed using Shapiro-Wilk and Kolmogorov-Smirnov tests. The homogeneity of the variances were also tested using Loon test. Independent t-test was used to compare the mean of these traits in two groups. Considering the repeated measurements, the repeated analysis of variance was used for quantitative variables. Survival analysis methods such as Kaplan-Meier were used to compare the duration to recovery. To adjust the confounding variables, covariance analysis and multiple regressions were used. SPSS V.18 was used to analyze the data, with the significance level of 0.05 in all tests.
Results

During the study, 305 patients referring to the cutaneous leishmaniosis treatment center in Gonbad-e Kavos, in 2015 were studied, of which 100 patients were included in the study based on the inclusion and exclusion criteria. The patients were randomly assigned to two groups of intervention and control, in which 55 patients received A. Vera gel and 45 patients received placebo. The distribution of the patients in the two groups was similar in terms of the mean age (p= 0.168), weight (p= 0.152), placebo lesions on the body (p= 0.382), treatment regimen (p= 0.728), size of the largest diameter of the wound (p = 0.022) and the largest induration diameter (p= 0.014) at the start of treatment.

However, the two drug and placebo-receiving groups were not similar in terms of the gender variable (P= 0.04) and the mean duration of the lesion (the longevity of the lesion when referring). The two drugs and placebo groups were similar in terms of the mean duration of treatment.

Considering the rate of healing in the placebo group, 17 patients (43.6%) were fully recovered, 16 (41%) had a relative improvement, 3 patients (7.7%) had mild recovery, 3 patients (7.7%) had deteriorated condition. While in the medicine group, 25 (53.2%) patients were completely recovered, and 36 (42.6%) had a relative improvement, 5 (4.3%) had mild recovery, and no patient had deteriorated condition. However, based on the P value of 0.216 in the chi-square test, it was determined that the recovery rate in the two groups was not statistically significant and it was the same.

Based on the treatment outcome in the placebo group, 24 patients (53.3%) were recovered, 8 (17.8%) had clinical resistance, 8 (17.8%) had failure, and 5 people (11.1%) were absent. While in the drug group, 36 patients (67.9%) were recovered, 7 (13.2%) had clinical resistance, 5 (9.4%) were failed, and 5 people (9.4%) were absent. Based on the P value of 0.471 in the chi-square test, it was determined that the results of the treatment of the two groups are similar. However, the gradient of reduction in the mean diameter of the wound in the placebo group was milder than that of the drug group (p= 0.012). The gradient of the mean wound diameter reduction in the placebo group was milder than that of the drug group and the longer the treatment is, the more pronounced the difference will be. As in the seventh week (p-value of 0.012), the decreasing trend in the mean diameter of the induration in the drug group was faster.
than the placebo group, the decrease in the mean diameter of the placebo group was milder than that of the drug group. According to the logarithmic test results, the decrease in the mean diameter of the wound in the two groups was statistically significant (p= 0.012).

**Safety profile**

No complication cases were reported in the drug group (Aloe Vera), even redness, inflammation or itching was not seen in patients. In the placebo group, a case of hives was reported in a child whose mother was treated with glucantine.

![Survival Functions](image)

**Discussion**

Limited studies were performed on the effect of A. Vera on leishmaniosis parasites, whose efficacy was proven, however, no study was performed on its effect on humans outside the laboratory. Nowadays, considering the general interest of people to treat with the medicinal herbs and the recommendation of the World Health Organization to use the local capacities and traditional medicine of each country to treat the native diseases of the region, the past texts were further taken into account. More than a thousand years ago, in the book "Hedayat Al-Motaalemin Fi Al-Teb", Guidance for Medicine Students (the available oldest Persian medical book) it was written about this disease: the bad wound (sore) transmitted from the mosquito bite. Akhaveini points out that he himself was affected by the disease, and the disease is difficult to cure. Among the treatments recommended in Iranian scientist's literature, Alov in or A. Vera can be repeatedly used on topical and even taken orally for treating this disease (15). Among the benefits of this
herb are the effects on wound healing, anti-fungal effects, reduced blood glucose or anti-diabetic effects, anti-inflammatory effects, immune system enhancement effects, and the protective effects of the stomach. Meanwhile, new studies indicated that the use of A. Vera gel, or even the whole leaf, can have an effect on intestinal absorption and even skin permeability [16]. Although the beneficial effect of using A. Vera in accelerating the wound healing was confirmed, it was also reported that the A. Vera gel can be used as an effective and inexpensive alternative to the treatment of bedsores, and articles were published on its beneficial effects on the various skin wounds, including breast fissure, burns, and even gastric ulcers (17, 18). However, no clinical trials were reported to examine the therapeutic effect of Aloe Vera plant on skin cutaneous leishmaniosis so far. The present study was based on the existing documents of traditional Iranian books on the therapeutic effects of topical compounds of A. Vera in the treatment of cutaneous leishmaniasis. According to the results of this work, although the local use of a combination of Aloe Vera had a positive effect on the outcome of the treatment and the rate healing the cutaneous leishmaniosis, these effects were not statistically significant. However, the use of A. Vera topically (Alovin) in the treatment of cutaneous leishmaniosis accelerated the process of reducing the diameter of the wound and its induration.

Concerning the effect of some herbal compounds on the treatment of cutaneous leishmaniasis, some work was performed previously, and some results were obtained, including in the study of Nilforoshzadeh (Isfahan, 2005-2007) at the end of the 12th week, 81.3% in the group treated with Nigella sativa extract in the honey base, and 64% in the control group were complete healed, and there was a significant difference between the two groups (p= 0.002) (19). In the study of Gholami (Isfahan), on the effect of cream containing 5% garlic in the treatment of cutaneous leishmaniasis, at the end of the third week, of 96 patients (18.75%) receiving the drug 18 people were healed completely, and 15 people(20 %) from the control group of 75 people were fully healed. There was no significant difference between the two groups (p = 0.9865) (20). In the study conducted by Jafari (Isfahan, 2008), at the end of the 12th week, 67.1%in the group treated with cassia fistula gel, and 41.4% in the control group were fully healed, and there was a significant difference between the two groups (p= 0.001), however, there was no statistically significant difference in terms of relative healing(21). In the study of Jafari (Isfahan, 2009-20100, on the effect of 5% Achillea millefolium gel, there was no significant difference between
the treatment group and the control group in terms of the complete and relatively healing at the end of the treatment period (4 weeks) \( (p = 0.35) \). (22)

Leishmaniasis is one of the most important zoonosis disease (23, 24). The use of medicinal and herbal plants such as aloe vera, which has antioxidant and healing properties, is recommended for the treatment of diseases (25-32). Studies have shown that medicinal plants due to their active ingredients and antioxidant compounds have beneficial effects on human health and have a therapeutic effect on various organs of the body and various diseases (33-45). Based on the above findings, it can be concluded that the use of 2% A. Vera gel has a positive effect on the rate of reduction in the size of cutaneous leishmaniosis wounds.

**Restrictions**

In the Iranian medicine texts, the treatments are in combination form. The combination of Alovin and vinegar or other compounds with certain ratios is recommended, however in this study, due to the restrictions, using just A. Vera was investigated. It is recommended in other studies to consider the therapeutic trends and combined medications based on Iranian medicine literature.

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**Conflict of Interest**

The authors declare that they have no conflict of interests.

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