Original Article

Anti Leishmanial Effect of *Plantago psyllium* (Ovate) and White Vinegar on *Leishmania major* Lesion in BALB/c Mice

Abdolali Moshfe[1,2], Keianoush Karami[1], Maryam Bahmani[1], Mohsen Naghmachi[1], Shahrbanoo Askarian[1], Abbas Rezaei[1], Roohallah Zare[1], *Ali Jamshidi[3]

[1]Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
[2]Department of Microbiology, School of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran
[3]Behbahan Faculty of Medical Sciences, Behbahan, Iran

***Corresponding author:** Dr Ali Jamshidi, Email: ajamshidi@behums.ac.ir

(Received 13 Mar 2019; accepted 18 Jan 2022)

Abstract

Background: *Leishmania major* is the etiologic agent of zoonotic cutaneous leishmaniasis in Iran, and glucantime injection is currently used for its treatment. This study aimed to evaluate the anti-leishmanial effect of topical *Plantago psyllium* and white vinegar in *L. major* infected BALB/c mice.

Methods: Thirty infected mice were divided into five groups as follows: Group 1: treated with the combination of ovata powder and white vinegar, Group 2: treated with glucantime, Group 3: treated with white vinegar, Group 4: treated with the combination of ovata powder and water, and Group 5: without any treatment. All the groups were treated for 18 days. Lesion size was measured, and final smears were prepared for microscopic examination.

Results: The findings indicated that the difference in the mean areas of the ulcers in all the groups before and after treatment was not significant, except for the second (glucantime) and third (vinegar) groups. Also, the results showed that in the first, second, third, and fourth group, 6 (60%), 4 (80%), 3 (60%), and 2 (40%) mice were healed, respectively. However, ulcers remained in all the five mice of the control group.

Conclusion: The combination of ovata powder and white vinegar has been traditionally used to treat leishmanial lesions in Iran. It seems the most anti-leishmanial effect is related to vinegar and supported by *Plantago*. The route of treatment with this combination is very simple and painless in comparison with injection. Thus, further studies on this issue could help to design more effective and easy-to-use drugs.

Keywords: Leishmaniasis; *Plantago psyllium*; Ulcer; Mice

Introduction

Leishmaniasis is one of the most important zoonotic diseases caused by more than 20 *Leishmania* species. This disease has a worldwide distribution and is transmitted by sandflies between animals and human hosts (1, 2). According to the World Health Organization (WHO) reports, leishmaniasis has a high mortality rate and is a health problem in endemic areas, especially in the developing countries (3, 4). Cutaneous leishmaniasis (CL) can be observed in the two forms of rural and urban in 97 different countries, and approximately 1.5 million new cases are reported throughout the world annually, with over 80% of the cases occurring in the developing countries (5, 6). Brazil, Iran, Afghanistan, and Sudan are the most infected countries (7).

Leishmania major is the main etiologic agent of zoonotic cutaneous leishmaniasis in some parts of Iran, and it has caused economic and health problems in these areas (8). For a long time, pentavalent antimonial drugs have been used as the first drugs of choice in the treatment of leishmaniasis. Currently, glucantime is used in many endemic countries because of its production as a generic drug. In

http://jad.tums.ac.ir
Published Online: March 31, 2022

some regions, mainly where resistance has developed, miltefosine, paramycin, and liposomal amphotericin B are gradually replacing the antimonials (9, 10). Nowadays, due to some factors such as side effects, drug resistance, excessive and economic costs of medical services, lack of easy access to these medications in some areas, researchers are looking for effective alternative drugs to reduce the side effects of CL treatment (11-13). One way to do so is the use of safe plant extracts in the treatment of the disease. The utilization of herbal extracts for the treatment of cutaneous leishmaniasis in many cities of Iran has a long history based on Avecina traditional medicine. According to the studies conducted in Iran (Refer to the review studies of Moghaddas et al. 2017, Oryan et al. 2015, and Soosaraei et al. 2017) in this field, more than fifty different local plants and extracts, oil, and powder of them (such as hydroalcoholic, dichloromethane, ethanolic, methanolic, and aqueous extract) have been used to treat cutaneous leishmaniasis, the findings of the mentioned studies showed that some of them like *Achillea millefolium* (Persian name: *bumadaran*), *Tanacetum parthenium* (Persian name: *babouneh*), *Nigella sativa* (Persian name: *siah daneh*), and *Satureja khuzestanica* (Persian name: *Marzeh khuzestani)* were exhibited anti-*Leishmania* effects activity (14-16). In the present study, *Plantago psyllium* (ovate) and white vinegar was used in the treatment on *Leishmania major* lesion in BALB/c mice, which was not previously reported in the anti-leishmania experimental studies.

Materials and Methods

In this experimental study, after disinfecting the leishmanial ulcer surface in mice, which were previously infected by the standard strain of *Leishmania major* (acquired from Shiraz School of Medicine with MHOM/64/IR/ER75 code), some of the ulcer margins were collected by a scalpel and dissolved in 6mL of normal saline. Then, by an insulin syringe, 0.2mL of the prepared solution (containing *L. major* amastigote) was injected into the tail of 30 male BALB/c mice aged 10 weeks old. This strain of mice was used because it is the most sensitive to *L. major*; the mice were purchased from Razi Institute in Shiraz in April 2016.

After infecting the mice, they were kept on special shelves under the same conditions. After three weeks, an ulcer appeared in the tail of all the mice. Then, to confirm the presence of the parasite (amastigote or Leishman body) in the lesions, Giemsa-stained slides were observed at 100× magnification. Afterwards, all of the 30 infected BALB/c mice were randomly divided into five groups.

The first group was treated with the combination of *Plantago psyllium* powder and white vinegar (10 mice). For this purpose, *Plantago psyllium* seeds were powdered by grinding. Then, 6.2g of *P. psyllium* powder was mixed in 25mL of white vinegar and a dough was prepared. A 4mm thick layer of the dough was used for treating the ulcers. The second group (as positive control), which consisted of five mice, was treated with 0.2mL of glucantime by intraperitoneal injection (According to the treatment method in many articles such as Nilforoushzadeh et al. 2008 and Taran et al. 2010). For the treatment of the third group (5 mice), a cotton ball soaked in 5ml of vinegar was used and placed on the wound for 2 minutes. Also, *P. psyllium* powder and water combination (4mm thick layer) were used for treatment in the fourth group (5 mice). Group five (5 mice) as the negative control did not receive any treatment. Before and after treatment (once every 72 hours), the ulcers' diameters were measured and recorded in all the groups by a caliper (ulcer size was calculated by measuring two diameters perpendicular to each other). All the groups were treated for 18 days, and at the end of the treatment period, smears were prepared for microscopic examination. The data obtained from microscopic

http://jad.tums.ac.ir
Published Online: March 31, 2022

observations, diameter changes, and lesions' healing were analyzed by SPSS version 20 using paired samples *t*-test. A P-value of less than 0.05 was considered significant.

Results

The findings of paired samples *t*-test indicated that the mean areas of the ulcers in all the groups were not significantly different before and after treatment, except for the second (glucantime) and third (vinegar) groups, (Table 1). The mean difference of ulcer area in all the groups was evaluated before and after treatment, and each group was compared separately with the second group (glucantime), which revealed a significant difference between the third, fourth, and fifth groups and the glucantime group (P< 0.05, Table 2). Also, in the first, second, third, and fourth groups, 6 (60 %), 4 (80%), 3 (60%), and 2 (40%) mice were healed, respectively. On the other hand, ulcers remained in all the five mice of the control group. The results showed a significantly (P< 0.05) smaller lesion size in the treated groups, especially in the vinegar and *P. psyllium* with water groups, compared to the control group. Anti-leishmanial effect of vinegar and glucantime were more than *P. psyllium* on under treatment mice.

Table 1. Comparison of the mean diameter (± SD) of lesions area in Balb/c mice due to *L. major*, before and after treatment in cases and control group

Group Name	Numbers	X±SD Before	X±SD After	Sig/NS
***P. psyllium* with Vinegar**	10	53.7±5.60	42.4±1.99	NS T= 0.36, DF= 9, p= 0.72
Glucantime	5	69±6.44	1.4±0.62	Sig T= 3.39, DF= 4, p= 0.028
Vinegar	5	23.5±4.13	9.2±5.8	Sig T= 3.3, DF= 3, p= 0.045
***P. psyllium* with Water**	5	38.7±2.47	34.5±9.47	NS T= 0.17, DF= 4, p= 0.87
Control	5	56.67±5.42	81.6±10.3	NS T= 0.64, DF= 4, p= 0.55

SD= Standard deviation, X= Mean ulcer area, DF= Degrees of freedom, NS= Non-significant, Sig= Significant, p= p value, and T= the t-value of the paired sample t-test

Table 2. The mean lesions diameter (± SD) of area in Balb/c mice due to *L. major*, in cases and control group compared with glucantime group, before and after treatment.

Group Name					Sig/NS
	Mean	SD	Mean	SD	
***P. Psyllium* with vinegar**	-30.11	44.98	-45.68	15.45	NS T= 1.21, DF= 9, p= 0.12
Vinegar	-60.20	41.12	-45.68	15.45	Sig T= -2.3, DF= 7, p= 0.038
***P. Psyllium* with water**	-4.23	23.54	-45.68	15.45	Sig T= -2.3 DF= 8, p= 0.038
Control	96.24	86.08	-45.68	15.45	Sig T= -2.14, DF= 8, p= 0.032

SD= Standard deviation, X= Mean ulcer area, DF= Degrees of freedom, NS= Non-significant, Sig= Significant, p= p value, and T=the t-value of the paired sample t-test

http://jad.tums.ac.ir
Published Online: March 31, 2022

Discussion

Cutaneous leishmaniasis is a great health problem in Iran. At the present time, no effective drug and vaccine for the inhibition of pathogenic leishmania spp and chemicals for eradication of its vectors is provided. According to the published research in the world, efforts are still ongoing to discover an effective treatment to cure cutaneous leishmaniasis with negligible side effects and low price. The current and standard medicine against cutaneous leishmaniasis is Glucantime, but it has many side effects. Therefore, traditional treatment of CL is a common habit of natives in many endemic areas in Iran. More than fifty different local plants are used to traditional treatment of cutaneous leishmaniasis lesions in Iran (14-16). Most of these herbal and traditional medicines (same as the findings of present study) showed anti-leishmanial effect, and also, they can play an effective role in healing wounds and finding an effective way to reduce injection pain and treatment costs. On the other hand, production of ointments containing herbal extract or essential oil are noninvasive method for treatment of cutaneous leishmaniasis (17-20).

The results indicated that there was a significant difference between the third, fourth, and fifth groups, and no significant difference between the glucantime group and the *P. psyllium* and vinegar groups. The findings of Hejazi et al. showed a significant difference in the means of lesion diameter before and after treatment in the control, yarrow, and thyme groups ($P< 0.05$). Also, paired *t*-test showed no significant difference in mean lesion diameter after treatment between treatment and glucantime groups ($P> 0.05$) (21). According to paired *t*-test, there was no significant difference in lesions' healing among different groups after treatment. Furthermore, the difference in the mean lesion areas before and after treatment in each group was compared with a positive control group (Group 2: treated with the glucantime) separately. Only the study by Westerhof et al. investigated the effect of *P. psyllium* on the treatment of cutaneous leishmaniasis. That study was aimed at investigating the effect of mucopolysaccharides on wound healing. The results of that study indicated that mucopolysaccharides limited the formation of scars (22). Other studies have also evaluated on the effects of herbal extracts on the treatment of CL. For instance, Ahmadi et al. examined the effect of garlic extract on CL, and their findings showed that the diameters of lesions were reduced by the hydrous extract of garlic over 30 days of treatment. However, the maximum reduction was achieved when mice were exposed to 10 days of vitamin A ointment, then treated for 45 days with garlic extract (23). Doroodgar et al. showed that *Artemisia sieberi* extract had no effect on the treatment of *Leishmania major* ulcers in BALB/c mice (24). Most studies on the treatment of CL using herbal extracts and natural products with anti-leishmanial activity so far indicate that none of these herbal extracts had a 100% effect on wound healing. Although some of these herbal extracts have been somewhat effective in the wound healing process and reduced the period of the disease (25-27).

Conclusion

The combination of *Plantago p.* powder and white vinegar has been traditionally used to treat leishmanial lesions in Iran. It seems the most anti-leishmanial effect is related to vinegar and supported by *Plantago*. The route of treatment with this combination is very simple and painless in comparison with injection. Thus, further studies on this issue could help to design more effective and easy-to-use drugs.

Acknowledgements

This article was financially supported by Yasuj University of Medical Sciences and ap-

http://jad.tums.ac.ir
Published Online: March 31, 2022

proved by the Ethics committee of Yasuj University of Medical Sciences (IR.YUMS.REC. 1397.023).

Ethical considerations

At the end of the treatment period, according to ethical considerations, all mice were killed and then buried.

Conflict of interest statement

Authors declare that there is no conflict of interest.

References

1. Sarkari B, Ahmadpour NB, Moshfe A, Hajjaran H (2016) Molecular evaluation of a case of visceral leishmaniasis due to *Leishmania tropica* in southwestern Iran. Iran J Parasitol. 11(1): 126–130.
2. Bates PA (2007) Transmission of *Leishmania* metacyclic promastigotes by phlebotomine sand flies. Int J Parasitol. 37(10): 1097–1106.
3. Yazdanpanah MJ, Banihashemi M, Mohammadi SM, Hatami Z, Livani F, Esmaili H (2015) Clinical features of Old World cutaneous leishmaniasis in elderly patients. Br J Dermatol. 172(2): 532–533.
4. Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, Arenas R (2017) Leishmaniasis: a review. F1000Res. 6: 750.
5. Moein D, Masoud D, Saeed M, Abbas D (2018) Epidemiological Aspects of Cutaneous Leishmaniasis during 2009–2016 in Kashan City, Central Iran. Korean J Parasitol. 56(1): 21–24.
6. Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J (2012) Leishmaniasis worldwide and global estimates of its incidence. PloS One. 7(5): e35671.
7. Salam N, Al-Shaqha WM, Azzi A (2014) Leishmaniasis in the Middle East: incidence and epidemiology. PLoS Negl Trop Dis. 8(10): e3208.
8. Norouzinezhad F, Ghaffari F, Norouzinejad A, Kaveh F, Gouya MM (2016) Cutaneous leishmaniasis in Iran: Results from an epidemiological study in urban and rural provinces. Asian Pac J Trop Biomed. 6(7): 614–619.
9. Sundar S, Chakravarty J (2015) An update on pharmacotherapy for leishmaniasis. Expert Opin Pharmacother. 16(2): 237–252.
10. Haldar AK, Sen P, Roy S (2011) Use of antimony in the treatment of leishmaniasis: current status and future directions. Mol Biol Int. 2011: 571242.
11. Zorbozan O, Harman M, Evren V, Erdoğan M, Kılavuz A, Tunalı V (2018) Infecting glial cells with antimony resistant Leishmania tropica: A new ex-vivo model. Mikrobiyol Bul. 52(1): 49–55.
12. Dujardin JC (2018) Epidemiology of leishmaniasis in the time of drug resistance (the miltefosine era). Drug Res Leishmania Parasites. 2018: 85–107.
13. Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R (2017) Drug resistance and treatment failure in leishmaniasis: A 21st century challenge. PLoS Negl Trop Dis. 11(12): e0006052.
14. Moghaddas E, Khamesipour A, Mohebali M, Fata A (2017) Iranian native plants on treatment of cutaneous leishmaniosis: a narrative review. Iran J Parasitol. 12 (3): 312–322.
15. Oryan A (2015) Plant-derived compounds in treatment of leishmaniasis. Iran J Vet Res. 16(1): 1–19.
16. Soosaraei M, Fakhar M, Teshnizi SH, Hezarjaribi HZ, Banimostafavi ES (2017) Medicinal plants with promising antileishmanial activity in Iran: a systematic re-

view and meta-analysis. Ann Med Surg (Lond). 21: 63–80.
17. Izadi S, Mirhendi H, Jalalizand N, Khodadadi H, Mohebali M, Nekoeian S (2016) Molecular epidemiological survey of cutaneous leishmaniasis in two highly endemic metropolises of Iran, application of FTA cards for DNA extraction from Giemsa-stained slides. Jundishapur J Microbiol. *9*(2): e32885.
18. Henry JC, Reedijk SH, Schallig HD (2015) Cutaneous leishmaniasis: recent developments in diagnosis and management. Am J Clin Dermatol. 16(2): 99–109.
19. Comini M, Flohé L, Jäger T, Oliver K (2013) Trypanosomatid Diseases: Molecular Routes to Drug Discovery. pp. 121–151.
20. Borazjani R, Aminnia S, Rastegarian M, Hosseini M, Ghanbarinasab Z, Ashkani-Esfahani S (2018) Effect of hydroalcoholic extract of *Arnebia euchroma* on the treatment of cutaneous leishmaniasis. J Clin Diagn Res. 12(8): 21–23.
21. Shirani-Bidabadi L, Mahmoudi M, Saberi S, Zolfaghari-Baghbaderani A, Nilforoushzadeh MA, Abdoli H, Moatar F, Hejazi SH (2009) The effectiveness of mix extracts of thyme, yarrow and propolis on cutaneous leishmaniasis: a comparative study in animal model (BALB/c). Tehran Univ Med J. 66(11): 785–790.
22. Westerhof W, Das P, Middelkoop E, Verschoor J, Storey L, Regnier C (2001) Mucopolysaccharides from psyllium involved in wound healing. Drugs Exp Clin Res. 27(5–6): 165–175.
23. Ahmadi-Renani K, Mahmoodzadeh A, Cheraghali A, Esfahani A (2015) Effect of garlic extract on cutaneous leishmaniasis and the role of nitric oxide. Iran J Med Sci. 27(3): 97–100.
24. Doroodgar A, Arbabi M, Razavi MR, Mohebali M, Sadr F, Tashakkor Z (2007) Effect of *Artemisia sieberi* extract on *Leishmania major* ulcers in BALB/c mice. FEYZ. 11(3): 52–56.
25. Rocha L, Almeida J, Macedo R, Barbosa-Filho J (2005) A review of natural products with antileishmanial activity. Phytomedicine. 12(6–7): 514–535.
26. Chan-Bacab MJ, Peña-Rodríguez LM (2001) Plant natural products with leishmanicidal activity. Nat Prod Rep. 18(6): 674–688.
27. Da Silva BJM, Hage AAP, Silva EO, Rodrigues APD (2018) Medicinal plants from the Brazilian Amazonian region and their antileishmanial activity: a review. J Integr Med. 16(4): 211–222.
28. Nilforoushzadeh MA, Shirani-Bidabadi L, Zolfaghari-Baghbaderani A, Saberi S, Siadat AH, Mahmoudi M (2008) Comparison of *Thymus vulgaris* (thyme), *Achillea millefolium* (yarrow) and propolis hydroalcoholic extracts versus systemic glucantime in the treatment of cutaneous leishmaniasis in BALB/c mice. J Vector Borne Dis. 45(4): 301–306.
29. Taran M, Mohebali M, Esmaeli J (2010) In vivo efficacy of gum obtained *Pistacia atlantica* in experimental treatment of cutaneous leishmaniasis. Iran J Public Health. 39(1): 36–41.