Comparison of Hyssopus Officinalis, Tussilage Farfara, Carum Copticum Extracts versus Systemic Glucantime in the Treatment of Cutaneous Leishmaniasis in Balb/c Mice

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Abstract

Leishmaniasis are transferred by sand flies belonging to the genus *Phlebotomus*. In this study, we evaluated the efficacy of Hyssopus officinalis, Tussilage farfara, Carum copticum plants and compared with systemic glucantime against cutaneous leishmaniasis in vivo. The studies were carried out on cutaneous leishmaniasis in inbreed mice to evaluate the effects of topical application of the ointment-based extracts two times daily for maximum of 20 days. A total of 45 mice were randomised into five groups (placebo group received the ointment base without the extract, systemic Glucantime as a reference group, Test groups: Hyssopus officinalis, Tussilage farfara, Carum copticum ointments) each including nine mice. NO production in macrophages was assayed. The result was suggestive that plants ointments were effective in production of nitrite but was not significantly more effective as compared with glucantime hence these plants are effective for treatment of cutaneous leishmaniasis in Balb/c mice.

Keywords: Invivo – Glucantime – leishmaniasis – Hyssopus officinalis
Introduction

Leishmaniasis is a parasitic disease transported by sand flies. Considering the prevalence of illness in Iran and many side effects associated with pentavalent antimony compounds use in its treatment, this study was designed. The disadvantages of the antimonials are their requirement for intramuscular or intravenous injection each day for 20-28 days, their toxicity, and the new and late development of resistance. Traditional treatment of CL is a common custom of natives in many endemic areas including many parts of Iran (1, 2). Recent investigations focused on plants have shown an alternative way to get a potentially rich source of drug candidates against leishmaniasis, in which effective alkaloids, quinones, iridoids, terpenes, indole analogues have been found. Moreover, topical treatment of CL is attractive compared with the systemic treatment because of the easy application, particularly in remote areas (3, 4). Hyssopus officinalis or Hyssop (Family: Lamiaceae) has soothing, expectorant, and cough suppressant properties. The plant also includes the chemicals thujone and phenol, which give it antiseptic properties. Antimicrobial, antifungal, antiprotozoal and anticancer effects of Hyssop extract have been reported. Tussilago farfara or coltsfoot (Family: Asteraceae) has been used in herbal medicine. Tussilago farfara leaves have been used in the traditional Austrian medicine internally (as tea or syrup) or externally (directly applied) for treatment of disorders of the respiratory tract, skin, locomotor system, viral infections, flu, colds, fever, rheumatism and gout. Carum copticum or Trachyspermum ammi (Family: Apiaceae) is administered in flatulence, atonic dyspepsia and diarrhea, and often recommended for cholera. In the Unani system, the herb is used as a drug to enhance the body's resistance, and is prescribed in amoebiasis, a parasitic infection of the intestines. It is a potent antimicrobial agent. The principal constituents of Bishop’s Weed oil are the phenols, mainly thymol and some carvacrol. Thymol is a powerful antiseptic and antifungal agent (5-9).

Materials and Methods

The Preparation of herbal extract (by maceration) and Parasites were done then topical formulation and ointment regimen design were done. In this study, we used outbred; female Balb/c mice aged 4–6 weeks and weighted 30–40 grams. The mice provided by Iran Pasteur Institute were randomized into five groups each including nine mice. The test groups were compared with those of placebo and control groups. Control group received Glucanite was supplied by Sigma and placebo group received the ointment base without the extract. This study was considered ethically approved by ethic committee of deputy of research of Iran University of Medical Sciences. About 6–8 week-old female Balb/c mice were then infected with 10^6 viable stationary-phase promastigotes through intradermal injection of parasites at the base of tail. After 30 days of inoculation of the Leishmania promastigotes, the treatment was started at the nodule site. Once a well-developed lesion was observed, the ointment was applied twice daily for a
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period of 20 days. To determine the amount of the ointment applied to the lesion, the ointment was weighted before application. It was found that a 200-mg amount of ointment was used per mouse per day using cotton applicator. After the end of the treatment period, mice in the experimental and placebo groups were followed for 1 month (10).

The Nitrit levels were measured by the Griess reaction, which is an assay for NO production. Peritoneal macrophages were harvested. Macrophages obtained from mice not treated and treated with different doses of the extracts and glucantime. The cells were cultured in DMEM medium containing 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 10% heat-inactivated fetal calf serum. Adherent macrophages were scraped from flask and washed with warm medium (25°C). Macrophages were counted and their viability was determined by trypan blue dye exclusion. Cells were cultured in 24 well plates (10^6 cells/ml, 1 ml/well) and incubated in 37°C, 5% CO₂ for 18 hr to adhere and non-adherent cells were removed. The Plants in absence and presence of 0.2 mM L-NMMA (iNOS inhibitor) were added to some wells. The supernatants were collected after 48 hr and NO production was determined by Griess reagent. Briefly, to 100 µl of culture medium, 100 µl of vanadium chloride (III) and 50 µl of Griess reagents [1:1 (v/v) of 0.1% naphthylethylene diaminedihydrochloride (NED) in H₂O + 2% sulphanilamide in 5% H₃PO₄] was added and incubated at 37°C for 40 min and the absorbance was read at 540 nm. Mean values were analyzed with a two way analysis of variance (ANOVA) and Student’s t-test. Differences between mean values were accepted as significant when p < 0.05. All statistical analyses were done using SPSS software, version 11.5(11-13).

Results

A nodule developed 3 to 4 weeks after the inoculation of 10⁶ promastigotes of L major into the base of the tail of a mouse. One to Two weeks later, the nodule transformed into an ulcer that was increased in size. Antileishmanial activity of plants extract was evaluated. After 20 days of treatment, twice daily, with the ointment base of plants extract the lesion size was studied up to the completion of the study. However more ability the herbals ointment to increase NO levels was shown (P < 0.05) in compare with GLU therefore there was no significant difference between them (P>0.05) in NO production. The Plants induced NO production but Carum copticum induced higher amount of NO not significantly. Our results showed that these plants to promote NO production by murine macrophages. The Effect of a 20-day period of topical treatment with ointment-based extract of herbals extract on cutaneous leishmaniasis Inbred mice infected with Leishmania major were shown in figure 1.

Discussion

Leishmaniasis is a group of tropical diseases caused by a number of species of protozoan parasites. Many therapeutic modalities have been used for the treatment
of cutaneous leishmaniasis. Pentavalent antimonials such as sodium stibogluconate, have been the base for therapy in the endemic regions because of its efficacy and cost effectiveness. The disadvantages of the antimonials are their requirement for intramuscular or intravenous injection each day for 20-28 days, their toxicity and the growing incidence of resistance in endemic and non-endemic areas. Due to the limited availability of effective pharmaceutical products, most people in areas where leishmaniasis is endemic depend largely on popular treatments and traditional medicines to alleviate the symptoms. In addition to the various methods already mentioned, the treatment of leishmaniasis following the traditional medical practices of different cultures depends heavily on the use of native plants (14, 15). To develop a suitable semisolid antileishmanial preparation, an ointment base of the extract was prepared. White soft paraffin (petrolatum) was selected as a typical oleaginous ointment base in view of its widespread use for many pharmaceutical ointments and lanolin was added to increase the hydrophillicity of the vehicle. DMSO is a well known penetration enhancer and was used to increase percutaneous absorption of the drug. Our study showed high efficacy of herbals against leishmaniasis in vivo. The plants used in this research 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. These plants have antiviral and antiparasitic activity such as essential oil of these against L.major. On the other hand, these plants are common table vegetable all over Iran and abundant in desert areas and access to these plants are very cheap and available. In our study Discontinuation of treatment did not lead to significant increase in the lesion size or parasite count during a 4-week period. In each test groups (10%), the lesions in 2 animals, out of 10, disappeared completely. The possible mechanism for this effect is through release of nitric acid and tumor necrosis factor from macrophages (10). In another study, peganine exhibited in vitro activity against both extracellular promastigotes as well as intracellular amastigotes residing within murine macrophages in L. donovani then P harmala seeds extract showed significant in vitro and in vivo antileishmanial activities (16). The results of this study mentioned above is in agreement with the results of our study. In this regard, medicinal plants seem to be promising candidates for drug discovery against leishmaniasis. In conclusion, to find the effective concentration and the mechanism of the effectiveness of plants, further investigations and extensive study with various concentrates of these plants essence are recommended also are evaluated against human cutaneous leishmaniasis as a randomized clinical trial.

Conflict of Interests
Authors have no conflict of interests

References


Fig 1: The NO production in the test, Glucantime and Placebo groups

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