Original Article

Effect of hydroalcoholic extract of *Echinacea purpurea* in combination with meglumine antimoniate on treatment of *Leishmania major*-induced cutaneous leishmaniasis in BALB/c mice

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Abstract

Context: Progressive resistance of *Leishmania* parasite to available drugs including, meglumine antimoniate, has been reported from various regions of the world, especially Iran. **Aims:** This study was conducted to evaluate the effect of hydroalcoholic extract of *Echinacea purpurea* in a combination therapy with glucantime in the treatment of cutaneous leishmaniasis caused by *Leishmania major*. **Materials and Methods:** Hydroalcoholic extract of *E. purpurea* was prepared from the plant. Amastigote form of *L. major* was inoculated to the tail base of thirty mice. After their tails became wounded, mice were divided into six groups. The first group was used as control and the second group received 100 mg/kg of *Echinacea* extract (orally). The third group was treated by meglumine antimoniate with dose of 20 mg/kg. Combination therapy was used for group four, five, and six where the mice received a different concentration of extract (100–200 mg/kg) and glucantime (10–20 mg/kg). The size of the cutaneous lesion on tail base was measured regularly. Findings were analyzed by SPSS software and using Kruskal–Wallis test. **Results:** The sizes of the lesion were increased in all mice of control group by the time. The mean size of lesions in mice receiving the extract and/or receiving the extract along with meglumine antimoniate was lower than those of control mice, but the differences were not statistically significant ($P > 0.05$). On the other hand, the differences between the group of mice which received meglumine antimoniate alone, and the rest of groups were statistically significant ($P < 0.05$). **Conclusion:** *E. purpurea* extract in doses which have been used in this study and combination with meglumine antimoniate was not much effective against *L. major* in BALB/C mice.

Key words: Cutaneous leishmaniasis, *Echinacea purpurea*, meglumine antimoniate combination therapy

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Introduction

Leishmaniasis is a group of diseases, which is caused by intracellular protozoan parasites, *Leishmania* and is

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transmitted through sand flies.[1] Cutaneous leishmaniasis which is known as Salak in Iran is a common disorder in several provinces of the country and is a major health problem in some provinces, such as Fars, Kerman, and Isfahan. It is estimated that about 12 million people are infected with *Leishmania* in various regions of the world, and 350 million people are at risk of leishmaniasis.[2]

First-line therapy in the treatment of this disease is pentavalent antimony derivatives, but drug failure and drug resistance are increasing in many regions of the world, especially in Iran.[3-5] In addition to different clinical responses of the patients to the treatment, side effects such as accumulation of drugs in tissues such as spleen and liver, myalgia, pancreatitis, cardiac arrhythmia, and hepatitis are the main problems of using these medicines in the treatment of leishmaniasis.[6] Therefore, it seems necessary to study and evaluate the new drugs for the treatment of leishmaniasis. One of the common methods regarding the appropriate treatment of this disease is the use of combination therapy. Various combination therapies have been recommended for this disease.

Simultaneous stimulation of the immune system, with stimulating compounds of the immune system, along with treatment with drugs might improve the treatment of this disease. One of the compounds which have attracted researchers' attention during the recent years is compounds from purple coneflower plant by scientific name, *Echinacea purpurea*. This plant which is known as the plant to strengthen the immune system is a plant of the Asteraceae family, and it had been considered a lot since a long time ago, and it has been used, widely, in traditional medicine. Recent studies showed that extracts of this plant increase the resistance of the body against the infectious diseases.[7-12]

It seems that combination therapy of the extract of this plant along with the first-line drug for the treatment of leishmaniasis (meglumine antimoniate) might improve the treatment of this disease. The extracts of *Echinacea* plant induce the stimulation of immune system by its immunomodulatory effect, and this might increase the effect of meglumine antimoniate. Considering the aforementioned points, this study was conducted to find out the effects of hydroalcoholic extract of *E. purpurea* in combination with glucantime on the treatment of *Leishmania major*-induced cutaneous leishmaniasis in BALB/C mice.

Materials and Methods

E. purpurea plant was provided by Zardband Company of Yasouj from medicinal herb garden in Yasouj, in the south of Iran. Dried *E. purpurea* plant, including leaf, aerial parts, and root were used to prepare the hydroalcoholic extract. The species of the plants was confirmed in the school of pharmacy of Shiraz University of Medical Sciences, and a herbarium specimens number (PM189) was assigned to it.

To prepare the extract, the plant was powered by the electric mill. Since the extract should be taken orally, ethanol at 30°C, without bitrex, was used to prepare the extract. Two liters of solvent (ethanol at 30°C) was added to 900 g of the powder of the plant and was stirred thoroughly. Then, another 2 L of solvent was added to the container of extracting after 24 h and finally, another 2 L of solvent (a total of 6 L) was added to the specimen for the third times after 24 h. In next stage, obtained extract was concentrated using rotary device after smoothing extract. To facilitate the conversion of extract to dry powder, the extract was concentrated in rotary device for several times and finally, it was reformed to dry powder by freeze dryer machine.

At first, thirty 5–6-week-old female BALB/c mice were obtained from the animal house of Shiraz University of Medical Sciences. *L. major* (MRHO/IR/75/ER) was used in this study. With consideration to this fact that available parasite in culture medium loses its pathogenesis due to various passages, the method of direct transmission of parasites from mouse to mouse was used to infect mice. Mice infection was done using amastigote form of the parasite. To do this, 0.2 ml of a suspension containing 4×10^5 amastigotes was injected in the top of mice's tail base by insulin syringe, subcutaneously. Lesions were developed after 25 days of inoculating parasites to the mice, in all mice. Mice were divided into six groups. The first group was control group which only received normal saline. The second group received the extract of *E. purpurea* plant orally with dose of 100 mg/kg. The third group was treated by meglumine antimoniate with dose of 20 mg/kg.

Combination therapy with the extract of *E. purpurea* plant along with meglumine antimoniate drug was used in fourth, fifth, and sixth groups where the fourth group received 100 mg/kg of extract and 10 mg/kg of meglumine antimoniate, the fifth group received extract with dose of 100 mg/kg, and meglumine antimoniate with dose of 20 mg/kg and the sixth group received the extract with dose of 200 mg/kg, and meglumine antimoniate with dose of 20 mg/kg. Meglumine antimoniate drug in all groups was used by intralesional injection of the drug for 20 days. Detection and confirmation of *Leishmania* infection in all of the studied mice group was based on parasitological detection of amastigotes in Geimsa-stained smear of the lesions. The size of lesion on tail base was weekly measured. The length of the major and minor axes of the lesion at the base of the mouse's tail perpendicular to each other were measured with a direct-reading Vernier caliper

and the area of the lesion in square millimeters was achieved by calculating ellipse area formula.

Statistical analysis

Kruskal–Wallis test were used to study the mean difference of the size of wounds in six groups of studied mice. Moreover, the trends of skin lesion changes in each mice group were compared, using ANOVA for repeated measures and *post hoc* multiple comparison test. The preintervention measures (time = 0) were used for adjustment of trend and grouping comparison. Significance was established for $P < 0.05$.

Results

The sizes of the lesion were increased in all mice of the control group by the time. Figure 1 shows the mean size of lesions in various periods of time in five groups of studied mice.

Statistical analysis of the mean size of lesions did not show any significant difference among groups of mice receiving the extract along with meglumine antimoniate with negative control group ($P > 0.05$). Also, comparing the trend of changes in each mice group, using ANOVA for repeated measures analysis, did not show a significant difference between groups of mice receiving the extract along with meglumine antimoniate with negative control group ($P > 0.05$). However, the differences between the group of mice which received meglumine antimoniate alone and the rest of groups were statistically significant ($P < 0.05$).

Discussion

Although pentavalent antimony derivatives are first-line therapy in the treatment of cutaneous leishmaniasis, but drug failure and drug resistance are increasing in many regions of the world. In some cases, other drugs such as pentamidine, amphotricine B, and paramomycin are used as the second option in cases of resistance to pentavalent antimony derivatives, despite their high toxicity for the patient.[13] Resistance to meglumine antimoniate in patients with leishmaniasis has been reported in Iran, in recent years.[4,14] It seems necessary to study about finding alternative medicines for the treatment of leishmaniasis.

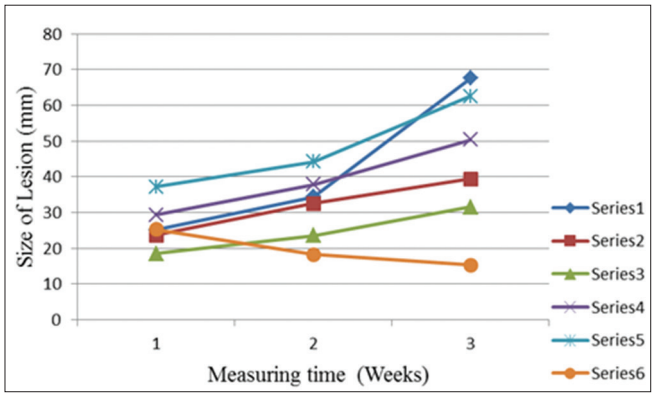

Figure 1: Mean size of lesions during three measuring times in different series of mice. Series 1: Received normal saline; 2: *Echinacea* 100 mg/kg; 3: *Echinacea* 100 mg/kg and meglumine antimoniate 10 mg/kg; 4: *Echinacea* 100 mg/kg and meglumine antimoniate 20 mg/kg; 5: *Echinacea* 200 mg/kg and Meglumine antimoniate 20 mg/kg; 6: Meglumine antimoniate 20 mg/kg

In this study, a significant difference was not obtained between the size of wounds in groups receiving the extract and drug and control group after studying mean size of created wounds in various groups of studied mice. This means that the combination of the drug with the extract has not improved the treatment of leishmaniasis in infected mice.

The reason for the lack of difference in the size of wounds in various groups of studied mice might be the interference of the extract of the plant with meglumine antimoniate drug in the treatment of the disease. Support for this statement comes from the observation that the group of mice which received meglumine antimoniate alone had smaller lesion size in comparison to the rest of the groups and that difference was statistically significant. This can be explained by the stronger effect of this compound alone, in comparison to the combination therapy. Ingredients of extract may inhibited the proper effect of meglumine in combination therapy.

Finally, inadequate consumed dose of the plant can be another reason in the lack of the effect of an extract of this plant in the treatment of cutaneous leishmaniasis in this study. Application of topical gel prepared with hydroalcoholic extract of *E. purpurea* (10 or 20%) for treatment of *L. major*-induced cutaneous leishmaniasis in BALB/C mice has not been encouraging. The authors suggested that dose of extract is one of the reasons for obtaining such unfavorable results.[15]

Taken together, in this study, the extract of *E. purpurea* plant at different concentrations along with meglumine antimoniate had not effectively been able to control the growth of *L. major* parasite in BALB/c mice. Using the extract of the plant in higher doses and also separating the nonhydroalcoholic compound of this plant is recommended in future studies with consideration to the importance of leishmaniasis and needs for new drugs in its treatment.

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Conflicts of interest

There are no conflicts of interest.

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