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Short Communication

Antileishmanial Effect of *Echinacea purpurea* Root Extract Cultivated in Iran

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Abstract

Due to the limited availability of effective pharmaceutical products and serious side effects of the available therapy for leishmaniasis, search for useful medicinal plants is necessary. Previous studies reported preventative effect of *Echinacea* against *Leishmania* infections through immunologic stimulation. Here we tested concentrated ethanolic extract of the root of *Echinacea purpurea* prepared from Zardband Pharmaceutical Co. for its direct leishmanicidal activity in *Leishmani*a culture. *Leishmania major* promastigotes (stationary phase) were cultured in different concentrations of the extract (0.5–125 mg/ml) for 30 min. Then the extract was removed and cell viability was determined during 120 h. LD_{50} for the promastigotes were determined as 22.3, 16.7, 3.66, 1.98 and 1.23 mg at 8, 16, 24, 48 and 72 h respectively. The results showed the irreversible leishmanicidal activity of the *E. purpurea*. Although all concentrations of the extract had antileishmanial effect, we suggest using this crude extract in concentrations of 50 mg/ml and more for application in related medicinal targets.

Keywords: *Echinacea purpurea;* Extract; *Leishmania major*; Leishmanicidal activity.

Introduction

Leishmaniasis is a group of tropical diseases caused by a number of species of protozoan parasites belonging to the genus *Leishmania*. This ailment affects some 12 million people in 80 countries and it is estimated that there are about two to three million new cases each year. At present, there also exists a population of 350 million of people under the risk of this infection (1).

The control of leishmaniasis remains a problem because no vaccine exists, and the available chemotherapy still suffers from potential toxicity and other side effects (2, 3). The rise in the rates of in vitro antimonial resistance due to intermittent drug exposure (4, 5) and the isolation of antimonial resistant patients with unresponsive cutaneous leishmaniasis necessitate the search for new agents for the treatment of leishmaniasis (6, 7).

In the recent years, there has been a growing interest in alternative therapies and especially in medicinal plants, as part of the WHO programs for finding better and low toxicity therapies for the treatment of leishmaniasis (8, 9).

Previous reports about the antileishmanial effect of E.purpurea are related to macrophage stimulation and its cytotoxicity induction

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(10, 11). Since there is no study on direct effect of *E.purpurea* on *Leishmania* and due to the critical importance of the control of leishmaniasis in Iran, the present research aimed at studying the antileishmanial activity of this extract.

Experimental

Plant material

The seeds of *Echinacea purpurea* (L.) Monch were brought from Hungary to Iran in 1993 by Dr. Reza Omidbeigi and cultivated in Zardband area in Northeast of Tehran. After preliminary studies on its growth and adaptation features, different extractions were prepared by Zardband Pharmaceutical Co. for pharmacological and other medicinal studies.

Extraction

Echinacea purpurea ethanolic extract was prepared from Zardband Pharmaceutical Co (Bach No. EPC04-L002-82) having a brown color, with a pH of 5.79, alcohol degree of 0 and bacterial count of 100 bacteria/g. Because of the change in dry weight in laboratory, the dry weight of the extract was determined according to the Hungarian Pharmacopia method, as 250 mg/ml (12).This extract was filtered with 0.22µ filter and then used in *leishmania* culture.

Parasite culture

The *L. major* strain, MRHO/IR/75/ER, was provided by Dr. S. Rafati (Pasture Institute of Iran).The parasite was kept in a virulent state by continuous passage in BALB/c mice (Pasture Institute of Iran). Homogenized spleens from the infected BALB/c mice were cultured in RPMI – 1640 media (Gibco-BRL) supplemented with 5% fetal bovine serum (FBS), 100 IU/ml penicillin, and 100 µg/ml streptomycin (Gibco-BRL). The promastigotes were harvested by centrifugation at 3000 rpm for 15 min at 4 °C.

Antileishmanial activity

The promastigotes of *L. major* (10^7 parasites/ml) were incubated in RPMI culture medium with 5% FBS in the absence or presence of 0.5, 2.5, 50 and 125 mg/ml of the crude root extracts from *E. purpurea* at 23 °C, in order to evaluate the parasite survival. Alternatively, after 30 min of the parasite growth in the presence of the crude root extract from *E. purpurea,* cells were centrifuged (3000 rpm, 15 min) and washed three times with Phosphate Buffered Saline (PBS) with a pH of 7.1-7.2 prior to resuspension in new culture medium without the plant extract, in order to evaluate the leishmanicidal or leishmaniastatic effect of the extract. Growth was determined by counting the cells during a 120 h period of incubation in vitro (2).

Statistical analysis

All experiments were performed in triplicate. The mean and standard error of at least three experiments were determined. For LD_{50} determination we used MINITAB® Release 14.20 demo version software downloaded from: http://www.minitab.com/products/minitab/14/demo/.

Other statistical analyses of the differences between the mean values obtained for experimental groups were done by using SPSS-Student's t test. P values of 0.05 or less were considered as significant.

Results and Discussion

The effect of concentrated ethanolic extract of the root of *Echinacea purpurea* on viability of *L. major*, causative agent of coetaneous leishmaniasis, was tested. LD_{50} for the promastigotes were determined as 22.3, 16.7, 3.66, 1.98 and 1.23 mg at 8, 16, 24, 48 and 72 h respectively. Figure 1 shows the time course of the viability of L.major in the absence or presence of different concentrations of *E. purpurea* extract. 125 and 50 mg/ml concentrations of this extract were able to kill 100% of the parasite after 48h (Figure 1). We find significant difference for 100% lethal effect between 50 mg/ml concentrations and more with lower concentrations ($P<0.05$).

Due to the lack of an effective and inexpensive chemotherapeutic agent for leishmaniasis and the serious side effects of the available drugs, discovering new drugs of herbal origin will have useful and interesting consequences (13, 15).

Previous lab studies in mice have shown that arabino-galactins from *Echinacea purpurea* provide a very good preventative effect against

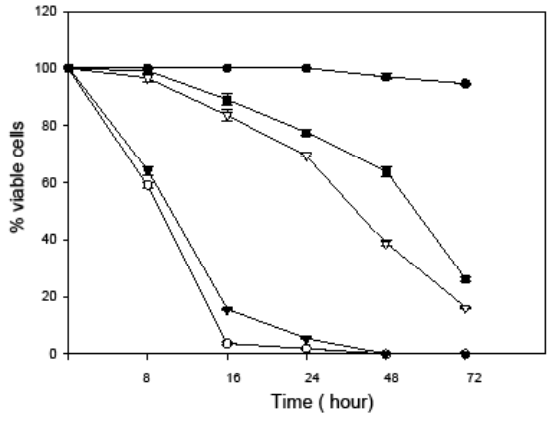

Figure 1. Time course of the viability of promastigotes of *L.major* in the absence or presence of different concentrations of *E.purpurea* extract. Cell viability (%) was calculated by [(L2/L1) × 100],where L1 is the number of viable control cells and L2 is the number of viable treated cells. (●) L.major promastigotes (control); (■) *L.major* promastigotes + 0.5mg/ml extract ;(▽) *L.major* promastigotes + 2.5 mg/ml extract; (▼) *L.major* promastigotes + 50 mg/ml extract; (○) *L.major* promastigotes+125 mg/ml extract. The values represent means of three independent experiments that were performed in triplicate. Bars represent standard errors.

lethal *Listeria* and *Leishmania* infections. These studies suggested that the antileishmanial activity was based on the immunostimulatory function and macrophage activation (10, 11).

In this work, we showed the irreversible leishmanicidal activity of the *E. purpurea* extract, by incubating the parasite for 30 min in a medium with the extract followed by culturing in a fresh medium. This suggests a metabolic injury that could not be reversed. Therefore the extract of *Echinacea* can be used both orally (for immunostimulation) and topically (for wound healing) in leishmaniasis. At present we are working on a project to find the active leishmanicidal compound(s) of the extract and the mechanism (s) of action.

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