The antileishmanial effects of *Lowsonia inermis* and *Cedrus libani* on *Leishmania major* promastigotes: an in vitro study

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Received: 10 January 2015 / Accepted: 22 June 2016 / Published online: 22 July 2016
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Abstract Leishmaniasis is a parasitic disease caused by different species of protozoan parasites belonging to the genus *Leishmania*. In this study, *Leishmania major* (MRHO/IR/75/ER) promastigotes were cultured at 23–25 °C in brain heart infusion (BHI) medium supplemented with 10 % heat-inactivated fetal bovine serum (FBS) and penicillin and streptomycin. Antileishmanial effects of *Lowsonia inermis* and *Cedrus libani* methanolic extracts (0.07, 0.15, 0.31, 0.62, 1.25, 2.5, 5, 10 mg/mL) on *Leishmania major* promastigotes were evaluated using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. All experiments were repeated at least three times. *Cedrus libani* methanolic extract did not show any activity while *Lowsonia inermis* methanolic extract inhibited the growth of promastigote forms of *L. major* in vitro after 72 h of incubation and had a 50 % inhibitory concentration (IC₅₀) of 1.25 mg/mL. The methanolic extract of *Lawsonia inermis* (henna) can be a promising antileishmanial agent in the future. Further experiments are needed for isolation of active fractions and identification of the active components of methanolic extract.

Keywords Antileishmanial effects · *Lowsonia inermis* · *Cedrus libani* · *Leishmania major* · In vitro

Introduction

Leishmaniasis is a parasitic disease with a wide range of clinical symptoms: cutaneous, mucocutaneous and visceral. They are caused by different species of protozoan parasites belonging to the genus *Leishmania* and transmitted through the bite of female sand fly (WHO 2004). Cutaneous leishmaniasis is endemic in 88 different countries. There are about 1.5 million new cases each year, with over 90 % occurring in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria (Old World) and in Brazil and Peru (New World) (Scarlsbrick et al. 2006). Cutaneous leishmaniasis (CL) occurs in two forms, zoonotic (ZCL) and anthroponotic (ACL). Zoonotic cutaneous leishmaniasis (ZCL) caused by *Leishmania major* is common in many rural areas of Iran (Mohebbali et al. 2004). Treatment for cutaneous leishmaniasis is unsatisfactory. Various forms of local treatment as heating, freezing, curettage and infiltration with antimonial compounds have their own advantages and disadvantages; however none of them is suitable for all forms of the disease (Berman 1988). For the chemotherapy of leishmaniasis, pentavalent antimony compounds including sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime) are the main treatments (Berman 2003). However, in recent years, the development of resistance to pentavalent antimony compounds has led to the introduction of newer antileishmanial drugs such as Miltefosine, Amphotericin B, Ketoconazole and Paromomycin (Guerin et al. 2002). However, there is limitation for their use due to long duration of therapy, toxic effects and high costs (Herwaldt 1999).

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The recent evidence has shown that an increasing number of Iranian patients with cutaneous leishmaniasis, particularly *L. tropica*, do not respond to meglumine antimoniate, the first line of treatment for leishmaniasis in Iran (Hadighi et al. 2006; Mohebbi et al. 2007). Many people who live in areas where leishmaniasis is endemic rely on traditional medicine systems for treatment, which depends heavily on the use of native plants (Chan-Bacab and Pena-Rodriguez 2001).

The recent studies showed that the plant extracts such as *Peganum harmala*, *Gossypium hirsutum*, *Ferula assa-foetida*, *Artemisia aucheri*, *Berberis vulgaris*, *Nigella sativa*, *Eucalyptus camaldulensis* and *Pistacia khinjuk* have been used against cutaneous leishmaniasis and the plant-derived components and plant extracts had the less side effects, low cost and high availability (Mirzaie et al. 2007; Barati et al. 2014; Mahmoudvand et al. 2014; Nosratabadi et al. 2015; Ezatpour et al. 2015).

Lowsonia inermis is commonly known as Henna belonging to family Lythraceae. It is abundantly available in tropical and subtropical areas. In several studies, antimicrobial activity including antibacterial, antiviral, antimycotic and antiparasitic activities of this plant have been proven (Tripathi et al. 1978; Riffel et al. 2002; Dinesh Babu and Subhasree 2009).

Cedrus libani (Cedar of Lebanon, Lotus) exists naturally in Syria, Turkey and Lebanon. The antimicrobial activity of *C. libani* was investigated (Digrak et al. 1999; Kizil et al. 2002).

In the present study we used the methanolic extracts of *Lowsonia inermis* and *Cedrus libani* to evaluate in vitro antileishmanial effects against *Leishmania major* promastigotes.

Materials and methods

Parasites and culture

Leishmania major (MRHO/IR/75/ER) promastigotes were cultured at 23–25 °C in Brain Heart Infusion (BHI) medium supplemented with 10 % heat inactivated fetal bovine serum (FBS) and penicillin and streptomycin (Evans 1989).

Methanolic extracts of *Lowsonia inermis* and *Cedrus libani*

The leaves of the plants were dried in room temperature and powdered. The methanolic extracts were obtained by maceration of the crude plant powders with methanol/water (90/10) for 2 days in room temperature. The extracts were then filtered and dried under reduced pressure at temperature below 45 °C.

Antileishmanial activity assays (MTT assay)

In this study, antileishmanial activity of *L. inermis* and *C. libani* methanolic extracts were performed using the method described by Mirzaie et al. (2007). The reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) to insoluble formazan has been employed as a parasite viability parameter in response to extracts.

A stock solution of MTT was prepared by dissolving MTT in phosphate-buffered saline (PBS) at 5 mg/mL and storing in the dark at 4 °C for up to 2 weeks before use. For the antileishmanial activity assays, 100 µL/well of the culture which contained 2.5×10^6 promastigotes per ml was seeded in 96-well plate. Then, 10 µL/well from various concentrations of Lotus and Henna methanolic extract (0.07, 0.15, 0.31, 0.62, 1.25, 2.5, 5 and 10 mg/mL) were added to duplicate wells and plates were incubated for 72 h at 22 °C.

The first well of 96-well plate was as a positive control well which contained of 100 µL medium containing 2.5×10^6 promastigotes per ml and the second well was as a negative control well which contained of 100 µL culture which contained 2.5×10^6 promastigotes per ml and DMSO 5 %. For every extract a row of 96-well plate was as blank which contained of 100 µL culture medium with different concentration of extract. At the end of incubation, 10 µL of MTT was added to each well and plate were incubated for 3 h at 25 ± 1 °C. After addition of MTT to parasites incubated with extracts, the reaction was stopped by the addition of 100 µL of 50 % isopropanol and 10 % sodium dodecyl sulfate. The plates were incubated for an additional 30 min under agitation at room temperature. The relative optical density (OD) was determined at 570 nm using an ELISA reader. All experiments were repeated at least three times.

Data analysis

The results of each experiment were analyzed by the method described by Bansal et al. (2004). The percentage of non-viable organisms which failed to metabolize MTT and therefore did not produce the formazan product was determined by applying the following formula:

Percentage of non-viable organisms or inhibitory percentage at each compound concentration = $100 - (\text{Test OD} - \text{Blank OD} / \text{Control OD} - \text{Blank OD}) \times 100$.

Results

The methanolic extract of *Cedrus libani* did not show any antileishmanial activity while the methanolic extract of *Lowsonia inermis* (Henna) inhibited the growth of

Fig. 1 The viability of *L. major* promastigotes in the presence of various concentrations of Lotus and Henna methanol extract

Fig. 2 Optimal density caused by the antileishmanial activity of different concentrations of Henna and Lotus methanolic extracts on the in vitro growth of *L. major* promastigotes

promastigotes forms of *L. major* after 72 h of incubation and had a 50 % inhibitory concentration (IC_{50}) of 1.25 mg/mL. The viability percent of *L. major* promastigotes in the presence of various concentrations of Lotus and Henna methanol extract are presented in Fig. 1. Also, the reducing optical density, caused by the antileishmanial activity of different concentrations of methanolic extract on the in vitro growth of *L. major* promastigotes, are presented in Fig. 2.

Discussion

The perspectives for the cure of leishmaniasis are still uncertain. *Leishmania* parasites are protected inside the macrophages, the compounds that are toxic to the parasite are also toxic to the host (Carvalho and Ferreira 2001). There is still no effective and cheap drug for the treatment of leishmaniasis. Although pentavalent antimonial compounds are the first-line of treatment for leishmaniasis and

amphotericin B and pentamidine are the alternative drugs, but all of these have serious side effects, high cost and drug resistance has been reported. Therefore, new drugs are urgently required. Natural products and plant extracts are valuable sources for the treatment of important tropical diseases caused by protozoans (Wright and Phillipson 1990). In the past decade, different plant-derived components and plant extracts have showed antileishmanial activity: a sesquiterpene lactone isolated from *Tanacetum parthenium* (Tiuman et al. 2005), a triterpenoid saponin extract from *Maesa balansae* (Maes et al. 2004), an indole alkaloid from *Peschiera australis* (Delorenzi et al. 2001) and a methoxichalcone from *Piper aduncum* (Caio et al. 1999). These studies have demonstrated that medicinal plants are the sources of new compounds for the development of novel antileishmanial agents. Plant-derived components and plant extracts have less side-effects, low cost and high availability. A large percentage of the world's population has no access to conventional pharmacological treatment (Rates 2001). However, even though these products have been traditionally used, there is no guarantee for their safety (Edzard 1998). For that reason, scientific investigation into the efficacy and safety of traditional medicine is needed.

In the present study, MTT assay was used to investigate the antileishmanial effect of Lotus and Henna methanolic extracts on the in vitro growth of *L. major* promastigotes. Several studies have shown that Henna has antibacterial, antiviral, antimycotic and antiparasitic activities. The antibacterial activity of the natural naphthoquinone products and their derivatives has been investigated (Riffel et al. 2002). The leaves of *Lawsonia inermis* were found to exhibit strong fungitoxicity where naphthoquinones were found to be the active factor (Tripathi et al. 1978).

Several studies have been carried out on antileishmanial effects of plant extracts that showed a good activity against *L. major* promastigotes. Mirzaie et al. (2007) studied on antileishmanial activity of *Peganum harmala* extract on the in vitro growth of *L. major* promastigotes in comparison to a trivalent antimony drug. Their study showed that *P. harmala* extract and potassium antimonyl Tartrate inhibited the growth of promastigote forms of *L. major* in vitro after 72 h of incubation and had an IC₅₀ of 1832.65 ± 89.72 µg/mL and 17.87 ± 2.05 µg/mL, respectively. The study carried out by Barati et al. (2014) revealed that anti-leishmanial activity of methanolic extracts of *Gossypium hirsutum*, *Ferula assa-foetida* and *Artemisia aucheri* inhibited the growth of promastigote stage of *L. major* after 72 h of incubation. Furthermore, Nosratabadi et al. (2015) reported that both methanolic and aqueous extracts of *Eucalyptus camaldulensis* inhibited the growth of promastigote forms of *L. major* in vitro after 72 h of incubation. Ezatpour et al.

(2015) also demonstrated that *Pistacia khinjuk* extract had in vitro effectiveness against *L. major*.

Our results showed that *Lawsonia inermis* (henna) methanolic extract had proper antileishmanial activity on promastigotes of *L. major*, and at concentration of ≥ 1.25 mg/mL inhibited the promastigotes growth after 72 h of incubation.

As a conclusion, further experiments are needed for isolation of active fractions and identification of the active components of Henna methanolic extract. In subsequent study, it is recommended to evaluate the antileishmanial effects of Henna methanolic extract against *L. major* amastigotes in macrophage cell culture and animal model. After scientific investigation into the efficacy and safety of the methanolic extract of *Lawsonia inermis*, it can be used for treatment of cutaneous leishmaniasis in the future.

Acknowledgments We thank the Office of the Vice-Chancellors for Research of Shiraz University of Medical Sciences for financial support of the project 87-4105.

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