

Anti-Leishmanial Activity of *Pelargonium roseum* Essential Oil on Growth of *Leishmania infantum* Promastigotes

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ABSTRACT

Background and Objectives: Due to adverse effects of pentavalent antimonial treatment of visceral leishmaniasis using herbal medicines has received a lot of attention. In this study, we evaluated inhibitory activity of *Pelargonium roseum* essential oil on growth of *Leishmania infantum* promastigotes, and compared it with that of Glucantime.

Methods: Inhibitory effects of different concentrations (1, 2.5, 5, 10, 25, 50 and 100 $\mu\text{L}/\text{ml}$) of *P. roseum* essential oil on the growth of *L. infantum* promastigotes were studied by MTT assay after 24 and 48 hours. All the data were analyzed by one-way analysis of variance. The effect of the essential oil was later compared with that of standard dose of Glucantime.

Results: After 24 hours, different concentrations of the essential oil had no significant inhibitory effect on promastigotes of *L. infantum*. After 48 hours, all concentrations except for 1 $\mu\text{L}/\text{ml}$ showed significant anti-leishmanial activity. In addition, the lowest concentration that had significant inhibitory effect on *L. infantum* promastigote was 5 $\mu\text{L}/\text{ml}$.

Conclusion: The essential oil of *P. roseum* has favorable inhibitory activity on the growth of *L. infantum* promastigotes after 48 hours. This indicates the therapeutic potential of this plant for treatment of leishmaniasis.

Keywords: *Leishmania infantum*, *Pelargonium*, Promastigote, Meglumine antimoniate.

INTRODUCTION

Visceral leishmaniasis (VL) is the most severe form of leishmaniasis caused by *Leishmania infantum*. The annual incidence of VL is estimated between 200,000 and 400,000 worldwide (1). The disease is endemic in different parts of Iran, but around half of infections occur in northwestern Iran, especially in the districts of Meshkin-Shahr. Dogs are the main reservoir of *L. infantum*, but the infection has been also reported in cats and rodents (2-4).

In Iran, the first-line treatment for VL is chemotherapy with pentavalent antimonial compounds. Meglumine antimoniate (Glucantime, Sanofi) and sodium stibogluconate (pentostam, GSK) are the only drugs registered for treatment of leishmaniasis (5). Considering the numerous side effects of antimony therapy, many scientists seek effective alternative herbal drugs with lower toxicity and higher efficacy (6). Vomiting, weakness, myalgia, abdominal colic, diarrhea, skin rashes, hepatotoxicity, cardiotoxicity and pain during intramuscular injection have been documented as common side effects of antimonials (7).

Pelargonium roseum is a plant native to South Africa, North America and Europe, but also found in other parts of the world. Essential oil of *P. roseum* contains geranium, citronellol, alcohol, phenyl ethanol, mannitol and amyl alcohol that are thought to have anti-inflammatory, analgesic, hemostatic and astringent antidiarrheal properties (6). Effect of *P. roseum* essential oil on *L. major* promastigotes, fungi and ticks has been demonstrated, but there is no report on its effect on *L. infantum* (8-10). Several studies confirmed the anthelmintic activity and therapeutic effects of this plant on gastrointestinal parasites (11). Although several studies have investigated alternative natural drugs for treatment of leishmaniasis, no study has yet evaluated the anti-leishmanial potency of *Geranium* (12). In the present study, we determined in vitro effects of *P. roseum* extract on the growth and viability of *L. infantum* promastigotes and compared it with that of Glucantime.

MATERIAL AND METHODS

Leaves of *P. roseum* were collected from the Avicenna Herbarium of Islamic Azad

University, Science and Research branch, Tehran, Iran (IAUH). Essential oil of the plant was prepared by hydrodistillation from fresh leaves. Gas chromatography analysis was performed to reveal its chemical constituents. The essential oil was diluted in 2.5% dimethyl sulfoxide (DMSO), and different concentrations of the essential oil were prepared. Glucantime was purchased from Sigma Chemical Co. (St Louis, Mo.). *L. infantum* (MHOM/FR/87/LEM1098) was provided by the Parasitology Department of Tehran University of Medical Sciences. Promastigotes of *L. infantum* (ANKT201383) were cultured in RPMI-1640 (Sigma-Aldrich) and 10% inactivated fetal bovine serum (Sigma, Cat N: F7942) in sterile condition at 25 ± 1 °C. Motility and number of promastigotes (around 1×10^6 parasites/mL) in the medium were checked before MTT assay, which is used to measure cell viability. To compare the effects of *Geranium* and Glucantime, 500 μ L of medium with 1×10^6 cells/mL promastigotes was seeded into wells of a 24-well plate. Then 2.5 μ L of DMSO was added to all duplicate wells except for that of control and Glucantime. Then, various concentrations of *Geranium* (1, 2.5, 5, 10, 25, 50 and 100 μ L/ml) and 100 μ L/ml of Glucantime were added to the duplicate wells. The plate was incubated at 25 ± 1 °C for 48 hours. Primary two wells of the plate contained only 500 μ L of medium without parasite and DMSO as a blank control, respectively. Finally, optical density (OD) of all wells was measured at 570 nm by a spectrophotometer (ELISA reader) after 24 and 48 hours. All the above processes were repeated three times. Data were analyzed by one-way analysis of variance and t-test in SPSS software version 14.0 (SPSS Inc., Chicago, IL) with significance level set at 0.05. Finally, the anti-leishmanial potency of different concentrations of the *Geranium* extract was compared with standard dose of Glucantime.

RESULTS

After 24 hours, Glucantime significantly affected the viability of *L. infantum* promastigotes. However, none of the concentrations of the essential oil of *P. roseum* significantly affected the viability of *P.*

roseum after 24 hours. In addition, there was no significant difference between the inhibitory effect of concentrations of 2.5-10 $\mu\text{L/ml}$ and 25-100 $\mu\text{L/ml}$ after 24 hours (Figure 1).

After 48 hours, all concentrations of the

essential oil except for 1 $\mu\text{L/ml}$ significantly affected the growth of promastigotes (Figure 2).

In addition, the lowest concentration that had significant inhibitory effect on *L. infantum* promastigote was 5 $\mu\text{L/ml}$.

Figure 1- Effect of *P. roseum* essential oil on *L. infantum* after 24 hours compared with Glucantime and negative control

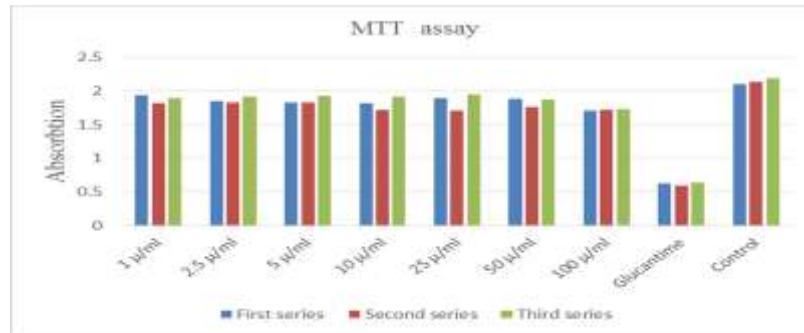
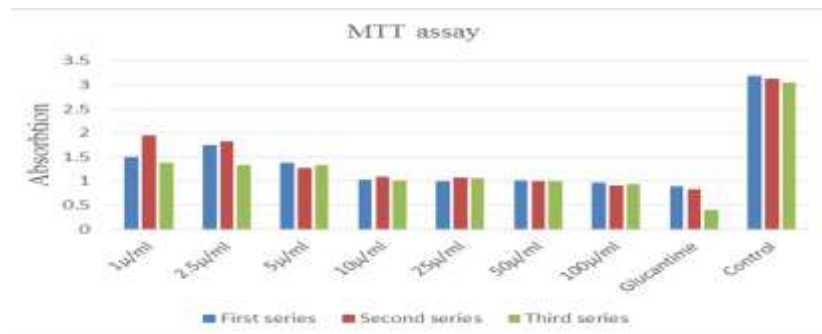


Figure 2- Effect of *P. roseum* essential oil on *L. infantum* after 48 hours compared with Glucantime and negative control



DISCUSSION

Leishmaniasis is an under-recognized disease and a major public health problem, especially in societies with poor socioeconomic status (13). High cost of treatment, resistance and side effects of drug therapy raised the need for treatment of leishmaniasis using natural herbal drugs (12). In the present study, we determined the anti-leishmanial activity of *Geranium* extract on *L. infantum* promastigotes in comparison with Glucantime. To our knowledge, no study has yet investigated the inhibitory effects of this plant on growth of *Leishmania* promastigotes. According to previous studies, different plants or herbal components have inhibitory activity against various species of *Leishmania* (12-14).

Mansour et al. investigated the inhibitory effect of ethanolic and aqueous extracts of *Vitis vinifera L.* leaves on *L. infantum* promastigotes. They showed that the ethanolic extract is more active than the aqueous extract because it contains higher concentrations of anthocyanins, which destroys cytoplasmic and nuclear membranes of promastigotes (13). Another study showed that *Thymus capitellatus* have anti-parasitic activity against *L. major*, *L. tropica* and *L. infantum* with 50% inhibitory concentration of 35-62 $\mu\text{g/ml}$. The extract of the mentioned plant affected mitochondrial membrane and caused cell-cycle arrest at the G0/G1 phase without causing cytotoxicity in mammalian cells (14). In the present study, all concentrations of the extract

exhibited Glucantime-like activity against *L. infantum* promastigotes after 48 hours. Although the mechanism through which the *Geranium* essential oil affects the parasites is not fully understood, it may be due to the lipophilic properties of the extract and its percolation in cell membranes (15). Furthermore, alcoholic extract of *Geranium* has shown antibacterial and immune-boosting properties. The anti-inflammatory role of the extract due to presence of citronellol and geraniol, which have been shown to suppress prostaglandin E2, redness, swelling and heat (16, 17). Although studies showed that several essential oils from plants such as *Croton cajucara*, white sacaca, basil, lemongrass and *Aloe vera* significantly increase nitric oxide production in the leishmanicidal process, the essential oil of *Geranium* suppressed abnormally elevated nitric oxide. Therefore, the essential oil of *Geranium* could be used as a side-effect-free anti-inflammatory drug (16). Crude essential oils not only have harmful effects on parasites, but could also have adverse effects on host cells. However, we did not evaluate the toxicity of this essential oil in

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CONCLUSION

The essential oil of *Geranium* show favorable in vitro inhibitory effects against promastigotes of *L. infantum* after 48 hours. These effects are similar to that of pentavalent antimony, the most commonly used drug for the treatment of leishmaniasis. Further studies are required to confirm our results and to investigate the in vivo effects of this essential oil.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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