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Anti-leishmanial activity of Pelargonium roseum Essential oil on growth of Leishmania infantum promastigotes in comparison to Glucantime

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ABSTRACT

Background and aims: Visceral leishmaniasis so called Kala-azar is one of the important health care problems worldwideSome herbal drugs are noticed because of toxicity and drug resistance less than pentavalent antimonials in the treatment of Kala-azar. In this study, anti-leishmanial activity of *Pelargonium roseum* Essential oil on the *in vitro* growth of *Leishmania infantum promastigotes* in comparison to Glucantime was studied.

Methods: Different concentrations of *Pelargonium roseum* essential oil in 1, 2.5, 5, 10, 25, 50 and 100 µL/ml on *Leishmania infantum* promastigote were studied with anti-leishmania activity assays at 24 hour and 48 hour at 570 nm wavelength. All the data were analyzed by SPSS (by ANOVA method) and anti-leishmanial potency of the oil extract of Geranium in different concentration were compared to standard dose of Glucantime.

Results: 1, 2.5, 5, 10, 25, 50 and 100 µL/ml concentration in 24 hour showed no significant difference of inhibitory on promastigotes of *L. infantum*. All concentrations except 1µL/ml in 48 hour MTT had resemble impact on the growth of promastigotes. 5 µL/ml concentration of *Pelargonium roseum* essential oil was significant difference effect on the growth of parasite (P= 0.043) in 48 hour.

Conclusion: Anti-leishmanial activity of *Pelargonium roseum* Essential oil on growth of *Leishmania infantum* promastigotes *wasn't* satisfactory in 24 hour MTT. But in 48 hour MTT it showed extremely effective against *L. infantum* growth inhibitory compared control group. This result indicated the effect of this herbal medicine on the parasite needs more time. *Pelargonium roseum* essential oil doesn't treat *Leishmania infantum* promastigote quickly. After 48 hour, 5 µL/ml concentration can be a suitable candidate in clinical trials.

Keywords: *Leishmania infantum*, *Pelargonium roseum*, promastigote, Glucantime

Original article

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INTRODUCTION

Visceral leishmaniasis (VL) is the most severe form of leishmaniasis, which is annually estimated a rate infection about 200,000 to 400,000 worldwide.[1] Iran is located in the Middle East where VL is seen in 14 out of 22 countries. VL in this region is caused by *Leishmania infantum* and is known as an endemic disease in different parts of Iran, such as northwest, southwest, east and south with a report of more than 2000 cases from 31 Iranian provinces and approximately 100 to 300 cases each year. According to some investigations which have done during last decades, around a half of infections occur in northwestern Iran especially in the districts of Meshkin-Shahr. The main considered reservoir is dogs; however the infection has been reported in cats and rodents in other researches.[2-4]

The first line drug for chemical treatment of VL in Iran is antimonials which are included in the National Essential Drug List for leishmaniasis. Meglumine antimoniate (Glucantime, Sanofi) and sodium stibogluconate (Pentostam, GSK) are the only drugs registered for leishmaniasis.[5]

Nowadays, with particular concern to numerous side-effects of antimony therapy, many scientists try to find effective herbal drugs with lower toxicity for body cells and higher efficacy[6]. Vomiting, weakness and myalgia, abdominal colic, diarrhea, skin rashes and hepatotoxicity, cardiotoxicity and pain during intramuscular injection have been documented as the common side-effects of antimonials.[7]

Pelargonium roseum is one of the native plants of south Africa, north America and Europe , but also it hasfostered in many parts of the world . Essential oil of *Pelargonium roseum* contain geranium, citronellol, alcohol, phenyl ethanol, mannitol and amyl alcohol which has approved multiple roles including anti-inflammatory, analgesic, hemostatic and blood-borne infectious disease, agents, astringent antidiarrheal.[6] There are several studies which have confirmed the therapeutic effects of this plant on gastrointestinal parasites and anthelmintic activity.[8]However, various researches have been screened alternative natural drugs to cure Leishmaniasis, but Geranium has not been studied for anti-leishmania potency in discovery programs.[9]

In present study determines the effect of oil *Pelargonium roseum* extract in comparison with Glucantime, pentavalent antimony, on the growth and viability of *Leishmania infantum* promastigotes in RPMI-1640 (Sigma-Aldrich) media and *in vitro* situation.

METHODS

Plant material and extraction: Essential oil (EO) was obtained by hydrodistillation from fresh leaves and Gas Chromatography (GC) analysis was performed to reveal chemical constituents of the EO. The oil was diluted in 2.5% dimethyl sulfoxide (DMSO), and different concentrations were provided.

Anti-leishmania drug:

Meglumine antimoniate (Glucantime), a pentavalent antimonial that was obtained from Sigma Chemicals (Munich, Germany). (Sigma Chemical Co., St Louis, Mo.).

Parasite and culture:

Promastigotes of Leishmania infantum (ANKT201383) were cultured in RPMI-1640(Sigma-Aldrich) and 10% inactivated fetal bovine serum (FBS, Sigma, Cat N: F7942) in sterile condition at 25 ± 1 °C. Moreover, the number of promastigotes with a count around 1×10^6 parasites/mL in medium and the motility of them were checked before MTT test.

Anti-leishmania activity assays (MTT assay):

For evaluation the oil extract of Geranium in comparison with Glucantime, 500 µL/well of the medium with 1×10^6 cells/mL promastigotes were seeded in 24-well flat-bottom plates. Then 2.5 µL/well of DMSO were added to all the duplicate wells except control and Glucantime wells. Afterwards, the various amount of Geranium including 1µL/ml, 2.5, 5, 10, 25, 50 and 100 µL/ml and Glucantime with standard dose of 100 µL/ml were added to the duplicate wells and plate was incubated in 25 ± 1 °C for 48 hour (hr). The primary two wells of plate contained respectively only 500 µL/well of the medium without parasite and DMSO as a blank and control in this test. Finally, the optical densities (OD) of all the wells were measured at 24 hr and 48 hr at 570 nm wavelength by a spectrophotometer (ELISA reader). All the process was repeated in three different dates.

Statistical analysis:

All the data were analyzed by ANOVA and the Student's t-test in SPSS software version 14.0 (SPSS Inc., Chicago, IL) with significance at P values of <0.05 and then the anti-leishmanial potency of the oil extract of Geranium in different concentration were compared with standard dose of Glucantime.

RESULTS

The results indicated a significant decreasing in the absorbance of the wells with Geranium compared to control and a inhibitory effect on promastigotes of *L.infantum* whereas the concentration of 2.5 to 10 µL/ml showed no difference to reduce the number of viable cells. This is noticeable that this group had a distinguished discrepancy with Glucantime well and other concentrations, so it was less effective at 24hr (P> 0.05).

Similarity, during the first 24hr, the second group of concentrations, 25 to 100 µL/ml, had no distinct difference in optical density and inhibitory percentage *in vitro* situation (P> 0.05). Furthermore, opposed to the former group the efficacy of the latter one was same as Glucantime on the growth of promastigotes.

Table 1: The significance of different concentrations of *Pelargonium roseum* Essential oil on growth of *Leishmania infantum* promastigotes

Different concentrations *Pelargonium roseum* Essential oil	MTT in 24 hour	MTT in 48 hour
1µL/ml	P> 0.05	P> 0.05
2.5 µL/ml	P> 0.05	p< 0.05
5 µL/ml	P> 0.05	p< 0.05
10 µL/ml	P> 0.05	p< 0.05
25 µL/ml	P> 0.05	p< 0.05
50 µL/ml	P> 0.05	p< 0.05
100 µL/ml	P> 0.05	p< 0.05

The analysis of obtained data by ANOVA at 48hr was evident that all the concentrations except 1µL/ml had resembled impact on the growth of rest of promastigotes. 5 µL/ml concentration of *Pelargonium roseum* essential oil were appreciable effect on the parasite (P= 0.043) in 48hr(table 1).

DISCUSSION

Leishmaniasis is one of the neglected tropical disease (NTD) and a major public health problem throughout the old and new world, especially in countries with poor socioeconomic conditions.[10] The high costs, resistance and side effects of chemical therapy for this disease have caused many researchers have been looking for natural herbal drugs as an alternative source to cure leishmaniasis worldwide.[9]

The main result of this study was to state the antileishmanial activity of oil extract of Geranium on *L.infantum* promastigotes in comparison with Glucantime, a pentavalent antimony, which was done *in vitro* situation. According to our knowledge, there are no pervious researches which have been investigated on this extract for inhibitory growth impact on promastigotes of *Leishmania*. Based on pervious investigations, different plant or herbal components possess inhibitory activity against various species of Leishmania parasite such as *L. major*, *L. tropica*, *L. aethiopica*, *L. amazonensis*, *L. braziliensis*, *L. mexicana*, *L. donovani*, *L. infantum and L. chagasi*.[9-11] The inhibitory effect of ethanolic and the aqueous extracts of *Vitis vinifera L.* leaves on *L. infantum* promastigotes were evaluated by Mansour et al in

2013. They showed etahnolic extract is more active than the aqueous one because of higher concentration in anthocyanins amount and consequently, more destruction of cytoplasmic and nuclear membranes of promastigotes.[10] Another study on a natural leishmanicidal agent, *Thymus capitellatus*, exhibited anti-parasite activity on *L. major*, *L. tropica* and *L.infantum* with 50% inhibitory concentration of 35 to 62 µg/ml via effect on mitochondrial membrane and cell-cycle arrest at the G(0)/G(1) phase without cytotoxicity on mammalian cells.[11] In present study, all the applied concentration ranging from 2.5 to 100 µL/ml have exhibited a same activity like Glucantime against *L.infantum* promastigotes after 48hr. However, the mechanisms of Geranium essential oil (GEO) are not fully known, but probably, lipophilic character leads a serious damage by percolating in cell membranes and change in the totality of cell structures and mitochondrial membrane, as well as other herbal essential oils containing hydrophobic molecules.[12] Furthermore, GEO with high concentration of alcohols (60-80 percent) has shown antibacterial and immune-support properties. Also, anti-inflammatory role of GEO is due to citronellol and geraniol which have been shown to suppress prostaglandin E2, redness, swelling, and heat.[13, 14] Moreover, in spite of several essential oils derived from plants such as *Croton cajucara*, *white sacaca*, *basil*, *lemongrass* and *Aloe vera* significantly increased nitric oxide (NO) production in the leishmanicidal process, GEO suppress NO as a pro-inflammatory when overproduced in abnormal situations. So, geranium oil could be used as a side-effect-free medicine instead of conventional anti-inflammatory drugs.[13] The toxicity of this oil extract have not been evaluated during the process in this investigation. It is considered the crude essential oils not only show harmful effect on parasite cells, but also could have adverse influence on the host cells. Although, recent researches have demonstrated that essential oils from *Croton argyropylloides*, *Artemisina annua*, *Menta villosa* and *Ligustim chuanxiong* are not toxic for infected animal cells with leishmaniasis.[9] Moreover, based on the results after analysis, even the lowest concentration of GEO (2.5 µL/ml) have a similar consequence to positive control with standard dose which it is not supposed to be damaging in this amount.

CONCLUSIONS

Overall, the results obtained from this research, GEO exhibited interesting efficiency against promastigotes of L.infantum in vitro after 48hr as well as pentavalent antimony which most used for the treatment of different forms of leishmaniasis while the dangerous side effects of this drugs have been confirmed. In addition, Geranium oil helps to speed up the healing process of leishmania ulcers and in fading the scars quickly by increasing blood circulation right below the surface of the skin. According to this reality that most of herbal medicines which have been studied as alternative only in vitro situation hence it is seriously recommend to be evaluated in clinical

trial and interpretation of their results into clinical practice.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

Authors' Contributions

Study concept and design and Critical revision of the manuscript: Mohaddeseh Abouhosseini Tabari. Analysis and interpretation of data: Bibi Razieh Hosseini Farash. Drafting of the manuscript: Elham Moghaddas, Mohammad Amin Ebrahimi, Nilofar Nabavi Mousavi. Statistical analysis: Mohammad Reza Youssefi.

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