Advanced Herbal Medicine, 2016; 2(2): 40-46.

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Antileashmanial activity of *Artemisia sieberi* essential oil against *Leishmania infantum in vitro*

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Received: 29/Jun/2016 Accepted: 20/Aug/2016

ABSTRACT

Background and aims: VL (Viseral Leishmaniosis) the second- most dreaded parasitic disease after malaria is currently endemic in 88 countries. *Artemisia sieberi* is native medicinal plants in Iran and their effects are scientifically proven to be effective on leishmaniasis. The aim of this study was to investigate antileishmanial effects of *A.sieberi* essential oil on *Leishmania infantum in vitro*. This is the first application of *A. sieberi* for treatment of *L.infantum*.

Methods: Promastigotes of *L. infantum* were treated by *A. sieberi* in 1, 5, 10, 15, 20 µg/ml concentration. MTT test ([3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide] was done in 48h after treatment to determine the effect on promastigote viability. The data were analyzed by SPSS, and using one-way ANOVA and Mann Whitney tests.

Results: Fewer than 15 µg /ml concentrations of *A. sieberi* essential oil were no appreciable effect on the parasite. A dose of 15, 20 µg/ml showed growth inhibitory on *L. infantum* in 24h/48h compared to control group ($P<0.05$).

Conclusion: The *A. sieberi* essential oil had antileishmanial effects against *L.infantum in vitro*. Therefore, they might be a reliable source for preparation of new drugs. More *in vivo* investigations are required to clarify details of effects of *A.sieberi* on leishmania spp and analysis of its natural components.

Keywords: *Leishmania infantum*, Artemisia, Promastigote, *In vitro* technique.

Original article

INTRODUCTION

Leishmaniasis including Cutaneous, mucocutaneous and visceral forms of clinical syndrome is one of the important health care problems in the world, especially in Iran. Because of a long time duration of treatment, being expensive, many side effects of drugs and resisted cases to conventional therapy, enormous efforts have been performed to replace herbal and new therapeutic strategies as alternative choices.

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Visceral leishmaniasis (VL) is a parasitic disease caused by *Leishmania infantum* and *Leishmania donovani* that are transmitted by the bites of phelbotomus in the old world and lutzomyia in the new world.[1,2] Visceral leishmaniasis is regarded as a protozoan zoonotic disease affecting incidence rate of 0.5 million and mortality rate of 60,000. Iran is one of the important foci of VL in the world that caused by *L.infantun* and transmitted by *Ph.larroussius*.[3] The available treatment options for VL have problems relating on efficacy, adverse effects, costs, resistance to treatment and side effects, especially in women and childbearing age.[4]

Artemisia sieberi with the common Persian name of ‘‘dermane’’, is a well-known medicinal plant that has been used in traditional medicine of the Middle East countries as an herbal medicine for treating various diseases.[5] Artemisia is included in the tribe Anthemideae and comprises over 500 species, which are mainly found in Asia, Europe and North America.[6] In the flora of Iran, the genus Artemisia has been introduced by 64 species.[7] that the most famous species were Turkish, Kermani and Caspian Artemisia. Artemisia species are frequently utilized for the treatment of diseases such as malaria, hepatitis, cancer, inflammation and infections by fungi, bacteria and viruses. It was previously reported that the aqueous extract and essential oil of *A. herba alba* have antileishmanial activity against *L. tropica* and *L. major* promastigotes.[8] In northern Africa, essential oil of this plant was very healing in *l. major* and *l. tropica* lesions.[9] Also, the aqueous extract of leaves of *A. indica* exhibited leishmanicidal activity (IC50: 430 µg/ml).[10] A lot of volatile molecules exist in essential oils and cause pleasant perfume in plants. Vast presence of monoterpenes and sesquiterpenes in Asteraceae are used as taxonomic markers for this family and especially Artemisia genus.[11] *Siberian Artemisia* contains bioactive substances including monoterpenes categories acid glycosides and 4-sezquitrepens derivatives of Oplonanon and germcran, derivatives bisabolene, salsolene ketones, camphor, 8,1 cineole of oxygenated monoterpene, sesquiterpene dehydroepiandrostrone, 1,8-cineole, b-thujon, thujon, alpha-dimethyl cyclopentane, carboxylic acid and camphor.[12] The combination of camphor, camphene, 1, 8-cineole, alpha and beta-tojone, alpha-pinnene are components of *Siberia Artemisia.*[13] Artemisia is the native plant in Iran and it grows in many provinces especially warm and dry areas. In addition, untreated leishmaniasis was reported 15% from glucantim drug and it is toxicity for heart, kidney and liver.[14]

The aim of this study were to study *A. sieberi* effect on leishmania and finding effective suitable doses of this herbal compound and compared to glucantime.

METHODS

Promastigotes of *L. infantum* (KT201383 strain) were maintained by RPMI-1640 medium supplemented with 10% bovine serum (FBS), 100 µg of Streptomycin/ml, and 100U of Penicillin/ml, with passage each 3 or 4 days at 26 ºC. Promastigotes of *L. infantum* were incubated at 26 ºC 48 hours. The parasites were not used after 3 *in vitro* passages. A negative control (with Glucantime: 150 mg/ml) and one DMSO + promastigotes were included in this study. Promastigotes are diluted to a concentration of 1.0×10^6 per ml of cultivation medium in a 24-well plate. *Artemisia sieberi* in appropriate concentrations is added to the experimental culture.

Standard *A. sieberi* essential oil was obtained from Barij Essence Pharmaceutical Company, Kashan, and Iran. Serial concentration was prepared (1,5,10,15,20 µg/ml) from standard stock solutions in DMSO as the solvent. *A. sieberi* inappropriate serial

concentrations (1,5,10,15 and 20 µg/mL) are added to the experimental culture. Overall, 5 different concentrations on five of 24-well plate containing 1.0×10^6 per ml *L. infantum* promastigote were evaluated. This survey was *in vitro* interventional study that describes the effectiveness of *A. sieberi* essential oil on *L. infantum* protozoa.

To find the effect on promastigote viability was assessed by MTT test ([3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide]. After 24h and 48h of treatment by *Artemisia sieberi*, MTT (1mg/ml, pH: 7.4) was added in each sample and it was incubated overnight in the dark at 25 °C. After that, isopropanol 50% and SDS (sodium 10% Dodecyl sulphate) were added and it incubated at 37 °C for 5h. Finally, samples were read at 540mm in a microplate reader. This test was repeated 2 times to make ensure repeatability.

All experiments were performed in duplicated. The data were analyzed by SPSS with one-way ANOVA and Bonfrony tests.

RESULTS

In the current study, the effect of 1,5,10,15,20 µg/ml concentrations of *Artemisia sieberi* were evaluated under *in vitro* conditions on promastigotes of *L. infantum*. It was showed at dose of 15 µg/ml (P=0.040), 20 µg/ml (P=0.027) that was extremely effective against *L. infantum* demonstrating growth inhibitory in 48h compared to control (Figure 1). 1,5,10 µg/ml concentrations of Artemisia essential oil were no appreciable effect on the parasite. Parasite was grown *in vitro* culture under various concentrations of this object with MTT measurement.

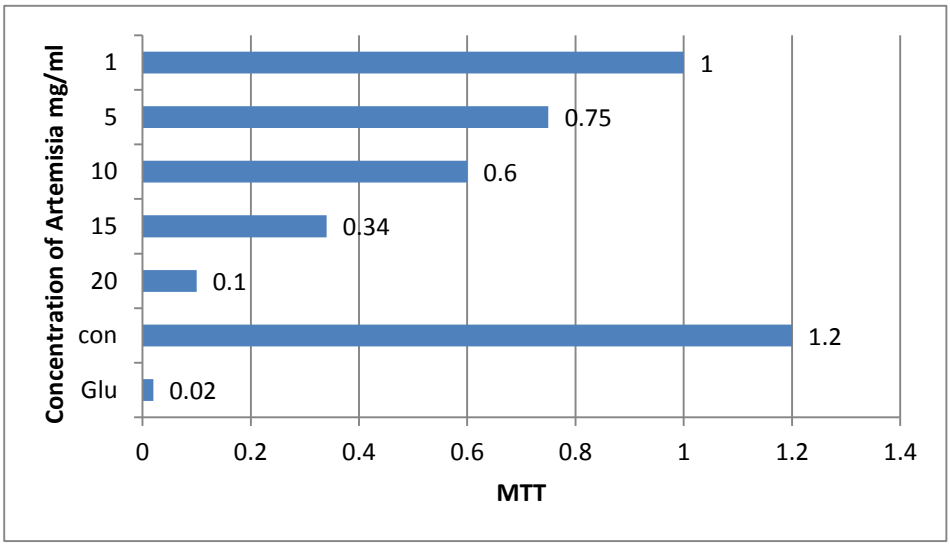

Figure 1: Growth curves of L.infantum culture were

Treated with different concentrations of Artemisia sieberi, glucantim and determination of parasite viability in MTT/48h (Con: control, Glu: Glucantime).

DISCUSSION

Screening of medicinal plants for antiparasitic activities is important for finding potential new compounds for medicinal and industrial purposes. In many cultures, some infectious diseases are known to have been treated with herbal remedies. The genus of Artemisia belongs to the family Asteraceae is presented by 34 species in Iran that are found wild all over Iran.[15] *A. sieberi* showed antifungal, antimalarial,

antiviral, antitumor, antihemorrhagic, anticoagulant, antioxidant, anti-hepatitis, and antiulcerogenic effects.[16-20] The WHO recommends artemisinin combination therapies as the first-line treatment for malaria.[21] Ethiopian *Artemisia* species, *A. absinthium* and *A. abyssinica*, also showed an activity against promastigote and axenic amastigote forms of two *Leishmania* strains, *Leishmania aethiopica* and *Leishmania donovani.*[22] Also, Artemisia is one of the most common plants used for removing intestinal helminthiasis and schistosomiasis.[23,24]

Results in the current study demonstrate 15 and 20 µg/mL concentrations of *A. sieberi* on growth inhibitory of *L.infantum* that was statistically significant compared to control groups (P=0.032). This result agrees with Tariko et al. that inhibitory effect concentration of *Artemisia absinthium* essential oil was 20 µg/mL on *L. aethiopica* and *L. donovani.*[25] It is important to find an herbal medicine with the least side effects from internal resources (native pharmaceutical drugs) that can economically compete with the available chemical remedies in the present pharmaceutical markets and to determine the effect of *A. sieberi* essential oil on *L. infantumn in vitro*. This is the first study of *A. sieberi* effect on the agent of visceral leishmaniasis. In another study 5,10,25,50, and 100 µg/mL concentrations of aqueous extract of *A. sieberi* and were tested on promastigotes of *L. major*, uninfected macrophages, and infected macrophages intracellular amastigotes of *L. major*, in comparison with the control groups. The effective dose was determined 25 µg/mL for *A. sieberi.*[26]

%1,%3 and %5 concentrations of *A. sieberi* hydro alcohol lotions wasn't observed in any treated mouse by Artemisia concentrates on *L. major* at the end of 30-day.[27] Inhibitory concentrations of essential oils of *A. sieberi* on *Fusarium moniliforme* and *Tribolium castaneum* showed 750 µM/L and 16.8 µ M/L.[28,29] Effectiveness against *L. major* promastigotes *A. sieberi* essential oils > *Pelargonium roseum* essential oils > glucantime was reported.[30] In another study ethanol, ethyl acetate, dichloromethane and hexane extracts of eleven Artemisia spp. native to Khorasan Province was checked out *L. major* promastigotes. All ethyl acetate extracts and *A.kulbadica* and *A.ciniformis* (IC50:0.025) have the most potent leishmanicidal activity.[31] Rostami et al. Studied *in vivo* efficacy of *Artemisia auchery* extract on *Leishmania major* cutaneous infection in the murine model and they introduced this extract was more effective on leishmaniasis.[32] The results of other studies on treatment effects of *A. sieberi* on leishmanial showed no toxicity even with the high concentration of the herbal extract, which confirms its minimal side effects.[33] Therapies were well tolerated, however; nausea, vomiting, dizziness, sleep disorders, and other neurological side effects were also reported in some studies.[34] The clinical use and the toxicity and teratogenicity of Artemisia and its derivatives, however, raise some queries and requirements for further studies. The oil of the species *A. absinthium* was also tested against eleven pathogenic bacterial strains with a minimum inhibitory concentration (MIC) of 0.14, 0.8 and 0.62 µL/mL, respectively.[35] It seems the extract of this plant is more effective on bacterial, fungal and insects rather than parasites. Due to their high volatility of this compound, it has fumigant activity that might be in high importance for controlling stored-product insects.

Artemisia.absinthium essential oil has been reported to show activity against the promastigotes and amastigote forms of *L.aethiopica* and *L. donovani.*[36] In another study, it was shown the leishmanicidal effect of essential oil from *Artemisia. annua* leaves against *L. donovani in vitro* and *in vivo.*[37] The action mechanism of *A. sieberi* against Leishmania is unknown. Furthermore, it is

important the kind of application *A. sieberi*. It was shown that ointment and intraperitoneal injection were effective than dropped essence of *A. sieberi* on the lesions in BALB/c mice. Even the group that treated with artemisinin ointment had better results in comparison with the group that treated with peritoneal injection form.[38] Dehkordi et al. study showed better therapeutic effects could be obtained treating visceral leishmaniasis by A. sieberi in 4 times per day, To find the effective concentration and the mechanism of the effectiveness of the drug, further investigations with fewer concentrates of *A. sieberi* essence are recommended.[39]

CONCLUSION

In conclusion, this study demonstrated that *A. sieberi* in 15 and 20 µg/mL has inhibitory effects on the *in vitro* growth on *L.infantum.* These results demonstrated the potential use of this compound as novel agents for the treatment of leishmaniasis. It seemed others compounds like carvacrol, thymol and linalool especially thymol have lower effective dose on leishmania spp. as under 1 µg/mL. Therefore it is more affordable and effective to produce a new drug from these compounds in comparison of *A. sieberi.*

CONFLICT OF INTEREST

Authors declare that there was no conflict of interest.

ACKNOWLEDGEMENT

We express our sincere thanks to Mashhad University of Medical Sciences, Iran for providing financial support for this study with Grant No. 941107.

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How to cite the article: Abouhosseini Tabari M, Youssefi MR, Moghaddas E, Ebrahimi MA, Nabavi Mousavi N, Naseri A. Antileashmanial activity of *Artemisia sieberi* essential oil against *Leishmania infantum in vitro.* Adv Herb Med. 2016; 2(2): 40-46.