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In Vitro Activity of Hyssopus Officinalis, Tussilage Farfara, Carum Copticum Extracts Against *Leishmania Major* in Iran

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Abstract

Leishmania is transmitted by sandflies that ingest the parasite in the amastigote phase resident within macrophages, then inoculate the promastigote phase into body hosts. The present study was conducted to evaluate the in vitro effects of alcoholic extract of Plants on *L.major*. The extract of aerial parts of plants were obtained by maceration. The in vitro experiments were performed on promastigotes to assess antileishmanial activity of the extracts using glucantime as

a reference. The extract of plants and glucantime solutions for biological testing were prepared in PBS at 0.05-0.1-0.2-0.4 μg/mL and 1μg/mL, respectively. All experiments were repeated at least three times in duplicate. For the extract of plants and glucantime, the concentration-response curve was plotted, from which IC50 values were determined also No and MTT assay were done. The different concentrations resulted in different optical densities or inhibitory percentages ($P<0.05$) so that extract of plants were effective against *L. major in vitro*. NO production the extract of plants showed significant in vitro antileishmanial activities.The Findings of this study indicate that these plants are effective against *L.major* in vitro.

Keywords: Antileishmanial activity, *Leishmania major*, Glucantime, Promastigote

Introduction

Cutaneous leishmaniasis is an endemic disease in many tropical and subtropical areas. Glucantime has been the mainstay for therapy in the endemic regions because of its efficacy and cost effectiveness. The disadvantages of the antimonials are their requirement for intramuscular or intravenous injection, their toxicity and the growing incidence of resistance in endemic and non-endemic regions. Recent investigations focused on plants have shown an alternative way to get a potentially rich source of drug candidates against leishmaniasis, in which effective alkaloid, phenol, tannin, flavonoid, thymol have been found (1-4). Medicinal uses of plants used in this research, Hyssopus officinalis or Hyssop (Family:Lamiaceae), Tussilago farfara or coltsfoot (Family: Asteraceae), Carum copticum or *Trachyspermum ammi* (Family: Apiaceae**)** have antiseptic properties, Antimicrobial, antifungal, antiprotozoal and anticancer (5-11). The objective of the present study was to determine the effect of plants extract compared with glucantime drug on the *in vitro* growth and viability of *Leishmania major* promastigotes.

Methods:

The *Carum copticum* seeds and *Hyssopus officinalis* and *Tussilage farfara* leaves were air dried at room temperature and kept in a dark amber-colored bottle until processed. The extract of aerial parts of plants were obtained by maceration. The *L. major* used in this study was the standard strain MRHO/IR/75/ER. The extract of plants solutions for biological testing and the drug were prepared in PBS at 0.05-0.1-0.2-0.4 μg/mL and 1μg/mL, respectively. *Leishmania major* promastigotes in late log phase were incubated in RPMI medium supplemented with 10% fetal calf serum, at an average of 10^6 parasites/ml. Parasites were

incubated, in duplicate cultures, with ascending concentrations of the extract solubilized in PBS. After 24 hours incubation period at 25°C, the surviving promastigotes were counted in a Neubauer's chamber. Half maximal (50%) inhibitory concentration (IC50) was determined as the concentration of the extract necessary to inhibit 50% of parasite growth. Negative controls treated by solvent (PBS) and positive controls containing glucantime were added to each set of experiments. The antileishmanial activity of extracts were evaluated in vitro against the promastigote forms of *Leishmania major* using a MTT 3-(4, 5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide)-based microassay as a marker of cell viability. The MTT assay used was based on that originally described by Mosma nn (1983) modified by Niks and Otto (1990) (12, 13, 14).The Griess reaction was adapted to assay nitrite. Briefly, nitrate was determined indirectly by the Griess assay. (15, 16).All experiments were repeated at least three times in duplicate. Mean values were analyzed with a two way analysis of variance (ANOVA) and the Student's *t*-test, with significance at P values of <0.05. (12, 16).

Results

The extract of plants and drug inhibited the growth of promastigote forms of *L. major in vitro* after 72 h of incubation, and had a 50% inhibitory concentration that were shown in figures. Details of the *in vitro* inhibitory effect of different concentrations of extracts and drug against *Leishmania major* promastigotes are presented in Figures. 1. and 2,3. Comparison IC50 of the extracts and drug was shown in figure.4. Also, details of reducing optical density, caused by the antileishmanial activity of different concentrations of extracts and drug on the *in vitro* growth of *Leishmania major* promastigotes, are presented in Figures. 5. and 6,7.

However, more ability the extracts to increase NO levels was shown ($P < 0.05$) in compare with GLU therefore there was no significant difference between extracts and drug. (P>0.05) in NO production. (Figure.8)

Discussion

There is a inclusive lack of effective and inexpensive chemotherapeutic agents for treatment of leishmaniasis. Natural products have potential in the search for new and selective agents for the treatment of important tropical diseases caused by protozoans (17).The results of this research show the antileishmanial activity of plants extract against *Leishmania major in vitro*. To our knowledge, based on a search of the literature, no studies have been conducted on the effects of (Carum copticum - Hyssopus officinalis - Tussilage farfara) plants extract on the *in vitro* growth of *Leishmania major* promastigotes (18,19).Previous *in vitro* experiments with *L. mexicana* promastigotes demonstrated that Triostam had a 50% lethal dose

of 20 µg of Sb (III)/mL. Other investigators have shown that trivalent antimonial compounds were highly toxic to different *Leishmania* species in the promastigote form at concentrations ranging from 1.58 to 35.00 µg of Sb (III)/mL (20-22). Several studies have shown that different protozoan infections have been susceptible to *P. harmala* extract in varying degrees (23, 24). The our results showed that there was no significant difference between plants extract and Glucantime (P>0.05) in MTT and IC50 test also NO production. In this study, the results showed that with a concentration increase of plants extract or drug, while the inhibitory effect on the growth of *Leishmania major* promastigotes will be increased, relative optical density will be decreased (19, 25, 26). Therapeutic evaluations for medicinal plants are essential because of the growing interest in alternative therapies and the therapeutic use of natural products (27). It is indicated that *L. major* infection naturally decreased NO induction in Balb/c mice as a result of amastigote action; but the researchers showed that artemisinin was not able to increase NO to combat parasite.It is concluded that artemisinin/glucantim action in CL wasnot associated with NO and CRP pathways(12) with attention to our results however more studies are needed to clarify other immunological parameters and used plant chemical composition in research.

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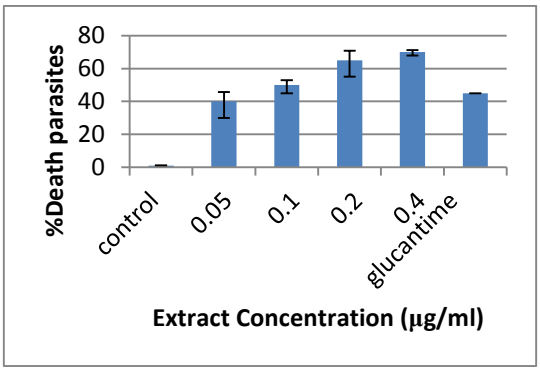

Fig1: Inhibitory effects of different concentrations of Tussilage farfara extract on the *in vitro* growth of *L. major* promastigotes after 72h.

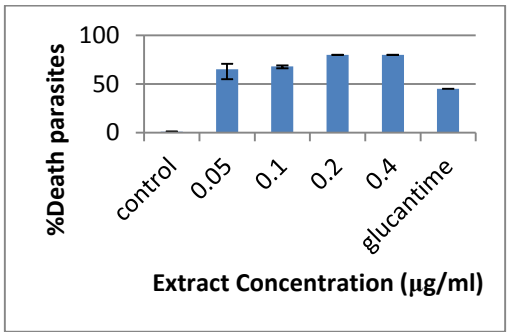

Fig2: Inhibitory effects of different concentrations of Hyssopus officinalis extract on the *in vitro* growth of *L. major* promastigotes after 72h .

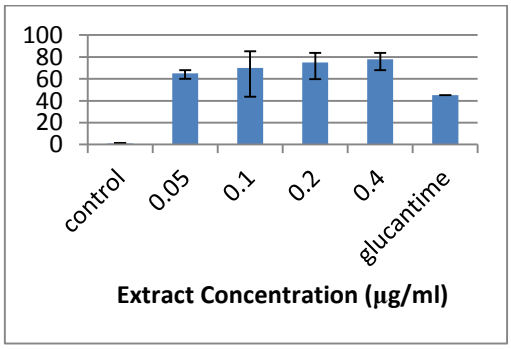

Fig3:Inhibitory effects of different concentrations of Carum copticum extract on the *in vitro* growth of *L. major* promastigotes after 72h .

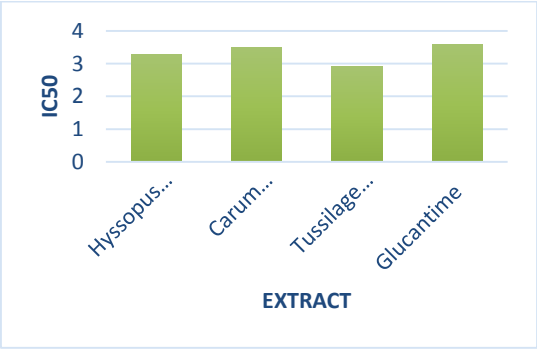

Fig 4: Comparison IC50 of the extracts and drug

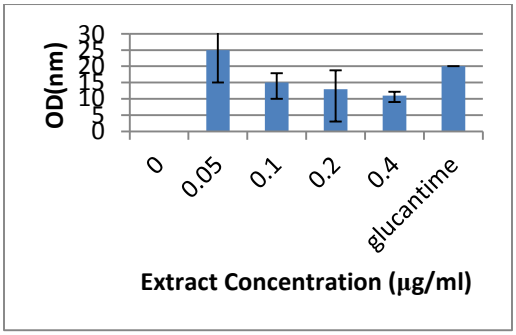

Fig 5: Reducing optical density caused by antileishmanial activity of different concentrations of Drug and Tussilage farfara.

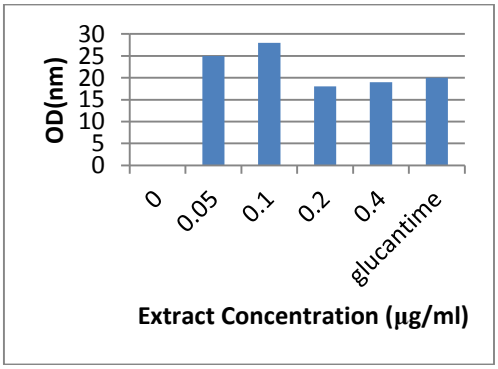

Fig6: Reducing optical density caused by antileishmanial activity of different concentrations of Drug and Hyssopus officinalis.

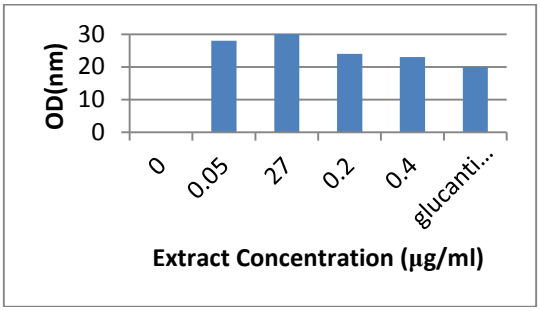

Fig 7: Reducing optical density caused by antileishmanial activity of different concentrations of Drug and Carum copticum .

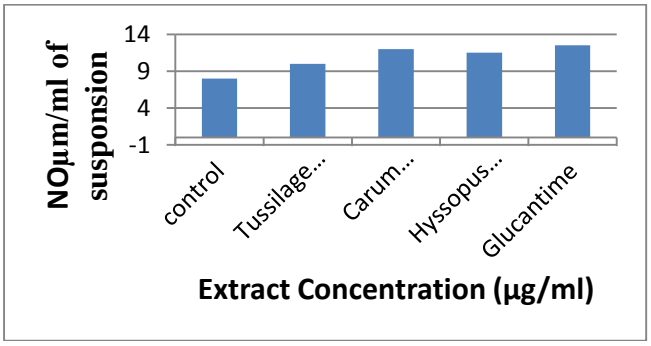

Fig 8 : NO production in the extract of plants and drug