Evaluation of anti leishmanial effect of *Agrostemma githago* on *Leishmania major* promastigote by MTT assay

Abstract

Introduction:

Leishmaniasis is a group of infectious diseases caused by various species of protozoa of the genus leishmania. Clinical symptoms of leishmaniasis include: Cutaneous, Mucocutaneous, Visceral and Diffused cutaneous leishmaniasis. At present, 12 million people are being affected by leishmaniasis, 350 million people are being threated around the world, and it is estimated that there are 2 million new cases, annually. Cutaneous leishmaniasis is common in Iran. The first-line drugs used for leishmaniasis treatment are pentavalent antimonials (Glucantime, Pentostam) which have many side effects including toxicity. In addition resistance to this drugs are increasing around the world. Second-line drugs such as Amphotricine B and Miltefosine have also side effects and expensive for patients. According to the anti-cancer and cytotoxic effects of *Agrostemma githago* extract, this study was conducted to evaluate the anti leishmanial effect of *Agrostemma githago* in comparison Glucantime on *Leishmania major* promastigotes by MTT assay.

Materials and methods:

A number of 2.5×10^6 *Leishmania major* promastigotes in BHI medium in stationary phase were added to each well of the 96 well culture plate. *Agrostemma githago* extract was added to the wells at different concentrations (1, 2, 3, 4, 6, 8, 12, 16 mg/ml). Glucantime was used as a control group. Cells were incubated for 48 hours in 24°C. After centrifuged

for 10 minutes in 4°C, supernatant were discarded and 50 µl of MTT were added. After 3 hours in 24°C and centrifuging in 4°C, the supernatant was removed and 100 µl of DMSO was added. After 15 minutes the plate was read by ELISA reader in 570 nm. Furthermore, Trypan blue assay was performed to evaluate agrostemma extract effect on *Leishmania major* promastigotes.

RESULTS:

MTT results displayed that increasing concentrations of Agrostemma extract could significantly reduce cell viability of *Leishmania major* promastigots compared to the control group ($p<0.05$). Also, inhibitory effect of Agrostemma extract was dose dependent. IC_{50} of the Agrostemma and Glucantime were calculated 0.365 and 71.01 mg/ml, respectively. Moreover, trypan blue assay showed that increasing concentrations of the extract reduced number of live *Leishmania major* promastigots.

Conclusion:

Aqueous extract of *Agrostemma githago* has stronger inhibitory effect than Glucantim (about 200 times) on *Leishmania major* promastigots. Althogh more expended studies are needed to evaluate its effect in “in vivo” conditions and on host cells.

Keywords: MTT assay, *Agrostemma githago*, *Leishmania major*