Evaluation of apoptotic pathway of *Agrostemma githago* extract on *Leishmania major* promastigotes

Abstract

Background and objective: Leishmaniasis is a group of infectious diseases caused by various species of protozoa of the genus leishmania. Clinical symptoms of leishmaniasis are different. Resistance to the first and second-line drugs is increasing in different areas of world. According to the anti-leishmanial effect of *Agrostemma githago* extract, this study was conducted to evaluation of apoptotic pathway of *Agrostemma githago* extract on *Leishmania major* promastigotes using caspase 3/7 enzymes and annexin V/PI activity.

Methods: After preparation of the extract, *leishmania major* promastigotes were treated with IC50 dose (0.4 mg / ml) of *Agrostemma githago* in BHI medium. The activity of Caspase 3 and 7 enzymes as well as staining by Annxin V/PA kit were measured by luminescence emmision and flow cytometry, respectively.

Results: The results of this study showed that the activity of Caspase 3 and 7 enzymes in the treatment group with a concentration of 0.4 mg / ml of *Agrostemma githago* was significantly increased compared to the control group which contains 1×10^7 of *Leishmania major* alone ($P < 0.001$). Treatment of *Leishmania major* promastigotes with IC_{50} concentration of *Agrostemma githago* extract and evaluation of induction of apoptotic pathway in these cells by annexin-V/ PI kit after 24 and 48 hours of treatment showed an increament in the population of early and late apoptotic cells compared with control group ($P < 0.001$). In addition, the percentage of late apoptotic subgroup was incresed in 48 hours treatment, in comparision to 24 hours of treatment. This accumulation at the late apoptotic pupulation was accompanied by a decrease of the cell populations in the early apoptotic subgroup ($P<0.001$).

Conclusion: Increased Caspase enzymes activity and the augmentaion of phosphatidylserine in the outer membrane of Leishmania promastigote parasites indicates an effective apoptosis induction following *Agrostemma githago* extract treatment.

Keywords: *Agrostemma githago*, *Leishmania major*, Caspase3,7 enzyme, Phosphatidylserin