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

Background & objective*:* Leishmaniosis is a group of infectious diseases that are caused by various species of *Leishmania*, and its clinical manifestations include: cutaneous, mucocutaneous, visceral and diffused cutaneous leishmaniasis. The prevalence of disease is high, and 12 million people are involved 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) which have multiple side effects such as drug toxicity. Moreover, parasite resistance to this drugs is rising around the world. Second-line drugs including Amphotricine B and Miltefosine have also side effects and expensive for patients. According to the cytotoxic effects of paraquat, this study was conducted to evaluate the anti leishmanial effect of paraquat, in comparison Glucantime on *Leishmania major* promastigotes by MTT assay.

Methods*: Leishmania major* was cultivated in BHI medium. A number of 2.5×10^6 promastigotes in BHI medium in stationary phase were added to each well of the 96 well culture plate. Paraquat was added at different concentrations (39, 58.6, 78.1, 117.2, 156.2, 234.3, 312.5, 468.7, 625, 937.5 and 1250 µg/ml). As a control group, Glucantime was used in different dilutions. The parasites were incubated for 48 hours in 24 ° C. The plate centrifuged for 10 minutes at 4 ° C and supernatant were discarded, 50 µl MTT was added into each well and, after three hours of incubation at 24 ° C, 100 µl of DMSO was added and OD was measured by a microplate ELISA reader (nm570). In addition, Trypan blue assay was performed to evaluate paraquat effect on *Leishmania major* promastigotes.

Results: MTT results showed that increasing concentrations of paraquqt could significantly reduce cell viability and number of *Leishmania major* promastigots compared to the control group ($p<0.05$). In concentrations of 468 µg/ml, all promastigotes were killed. In this study, the IC50 was 272/46 µg/ml. Trypan blue assay confirmed results of MTT assay.

Conclusion: Paraquat has strong inhibitory effect on *Leishmania major* promastigots. More expended studies are needed to evaluate its effect in "in vivo" conditions.

Keywords*: Leishmania Major, Paraquat, MTT assay*.