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# Effects of Selenium Red Nanoparticles on Leishmania Infantum, Cellular Apoptosis and INF-γ and IL-4 Cytokine Responses Against Visceral leishmaniasis

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**ABSTRACT**

Leishmania is an intracellular protozoan and causative agents of visceral leishmaniaisis [VL]. Leishmaniasis is considered as one the most important tropical disease by World Health organization [WHO][1]. Leishmanial drugs are costly, high toxic, frequently have unpleasant side effects. In addition, drug resistance exits in region of endemicity. Perviously using nanoparticles in treatment of leishmaniasis has been became more attractive.Several researches reported recently, selenium nanoparticle has developed as promising tool for treatment leishmaniasis progression [10,11] Antioxidants effects of selenium has been shown that protect phagocytic cells and surrounding tissues from attacking free oxidative radicals produced by the respiratory chain of neutrophils and macrophages during phagocytosis. There is one kind of nano red selenium demonstrated to have comparable bioavailability with selenite and also low toxicity rather than selenite for normal cells [23, 24]. Unique properties in biological pathways and low toxicity red nanoselenium are attracting more attention in scientific circle [10]. In the present study we described selenium red nanoparticle on leishmania infantum.



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## INTRODUCTION

Leishmania is an intracellular protozoan and causative agents of visceral leishmaniasis [VL]. Leishmaniasis is considered as one of the most important tropical disease by World Health Organization [WHO] [1]. VL causes significant mortality worldwide, constituting an important public health problem [1,2]. Leishmanicidal drugs are costly, high toxic, frequently have unpleasant side effects. In addition, drug resistance exists in regions of endemicity. Previously using nanoparticles in treatment of leishmaniasis has been became more attractive. Several researches Emerging technologies using metal nanoparticles have been recently adapted for the study of host- Leishmania interactions to describe in treatment of leishmaniasis [6].Nanosilver showed high antimicrobial activity and also it has been used against leishmania tropica [8,9]. It has been reported that nanosilver is a more effective that antimony, the first line srug against Leishmaniasis in most endemic countries [Inhibitory]. In addition, the nanoparticles are able to induce an antiproliferative effect on the Leishmania at metal concentrations lower than those used with anitmony [Inhibitory Nanogole particles have alsobeen used in treatment of cutaneous Leishmaniasis [7].Several researchs reported Recently, selenium nonparticle has developed as promising tool for treatment Leishmaniasis progression [10, 11]. The first enzyme characterized as selenium dependent was glutathione peroxidase cytosol. Selenium plays an important role in the balancing of Redox system, function system [12, 13, 14]. Antioxcieants effect of selenium has been shown that protect phagocytic cells and surrounding tissues from attacking free oxidative radicals produced by the respiratory chain of neutrophils and macrophages during phagocytosis [15 Selenium acts as a cofactor in glutathione peroxide enayme structure, which catalyzes the reduction of peroxides formed during fatty acids metabolism to prevent the damage of cell memberanes [16-20]. There is one kind of nano red seldnium demonstrated to have comparable bioavailability with selenite and also low toxicity rather than selenite for normal cells [23, 24]. The

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physiological role of selenium in the animal is determined by its participation as a cofactor of different enzymes: glutathione peroxidase cytosolic [c-GSH-Px], plasma glutathione peroxides [GSH-Px p-] phospholipid hydro peroxides. glutathione peroxides [GSH-Px-PH], glutathione peroxides gastrointestinal [GI-GSH-Px] lodotironina 5 "deiodinasee [IDI5"], thioredoxin, glutaredoxin [15,16,18].

Additionally selenium is a part of selenocysteine and selenomethionine proteins, the main amino acid constituent of these enzymes is selenocysteine, located in the active sites??? [it is not clear please clarify what is you mean [17].Unique properties in biological pathways and low toxicity red nanoselenium are attracting more attention in scientific circles [10]. A major challenge facing chemotherapy of leishmaniasis is failure of humeral and particularly cellular immune system. Therefore it is conceivable that nanoselenium can be useful in elimination of intracellular leishmania with in macrophages. In the present study we described selenium nanoparticles that were synthesize by a strain of *Klebsiella peumoniae* from selenium chloride with an average size of 245 nm[27]. The aim of this study is to determine the effects of selenium against *Leishmania infantum* in vitro and in vivo conditions for the first time. The inductions of apoptosis in the Leishmania cells by these nano particles were also exmined by flow cytometeric methods. Finally this study aimed to study about effects of various doses of nanoselenium against visceral Leishmaniasis in BALB/c mice.

Elemental selenium has been known to exist in various allotropic forms, as res amorphous form, black vitreous form, three [$\alpha, \beta, \gamma$) of red crystalline monoclinic forms and grey/ black crystalline hexagonal [also referred to as trigonal] form which is also the most stable form, and some more allotropes are being discovered [51-59]. Commerically, selenium is produced as a byproduct of copper refining. Industrially it finds use in electronics, glass, ceramics, steel and pigment manufacturing [60].

The photoconductivity of selenium found its use in a photometer by Siemens, 1875, a photophone by Graham Bell, 1880, an optophone by Fourniere d'Albe, 1912 and then talking films in 1921 [61]. Selenium was used in redtifiers until the industry found a cheaper and readily available alternative in silicone.

*Methods:*

Nanoselenium was provided [needs a brief describtion and then refer to [27]

*Cultured parasites:*

Leishmania infantum strains standard [MHOM/IN/80/IPH]-[gift from Prof. Rafati or somebody else?? In Pasteur Institue of Iran]. Pasteur Institue of Iran].

Promastigotes were cultured in completed RPMI1640 medium [GIBCO] supplemented with 10% hated fetal calf serum [Hi-FCS] [which company]and penicillin [100 IU/ml]and streptomycin [100 $\mu$g/ml] and incubated at 25˚C

*Promastigote assay:*

Stationary or log phase promastigote was harvested and suspendented in completed RPMI [$2\times10^6$ cell/ml]. The saple [100$\mu$l] was transferred in 24-wells tissue culture plate containing 100$\mu$l of completed RPMI 1640 medium and treated with serial dilutions of the selenium nanoparticles [1, 2.5, 5, 10, 25, 50, 100$\mu$g/ml] and incubated for 24, 48, 72 hours. The antileishmania activity of nanoselenium was evaluated by counting the alive parasites and were compared with control cultures.

*Colorimetric MTT method:*

The ability of cells in transforming the yellow terazolium crystals to blue color was measured using a modification of previously described MTT colorimetric method [28,29 Briefly, 100$\mu$l] of promastigotes [$2\times10^6$ cells/ml] was added in to 96-wells plates containing 100$\mu$l of RPMI1640 medium supplemented with 20% FCS. These cultures were repeated at least three times in triplicate wells. 200$\mu$l of promastigotes were cultured as control group. 200$\mu$l/well PBS was added around wells of plate to prevent the evaporation of well contents. The cells were incubated in presence seven dilutions of nanoselenium at $24\pm1^{\circ}C$ for 72 hours and then 20$\mu$l of MTT solution was added into each of wells.Plates were incubated again at $24^{\circ}C$ for 4 hours and then

centrifuged at 1000g for 10 minutes. Supernatant was aspirated gently and discarded. 100$\mu$l DMSO was added to each of Wells and finally The absorbance of these plates was measured by the ELISA reader system in at 540 nm. Testes using a modification a previously described method [1]. In brief, 100$\mu$l suspensions of L. infantum WT and H-line stationary phase promastigotes [$2.5\times10^5$] were incubated at 37$^{\circ}$C for 2 followed by incubation for 1 h in the presence of different concentrations.

*Macrophage cytotoxicity:*

For evaluation effects of nanoselenium on un-infected mouse Macrophages, we have used Inbred male BALB/c mouse was purchased from the Laboratory Animal of Razi institute of Iran. At the first 7 ml of RPMI medium [sigma] was injected in to peritoneum and macrophages were collected. After that the number of live macrophages was estimated. 100$\mu$l of macrophages with 100$\mu$l RPMI1640 medium were seeded in exposure to seven dilutions of nanoselenium. These cultures were maintained at 37$^{\circ}$C in the presence of 5% $CO_2$for 24,48,72 hours. After which the experiment was terminated direct counting was applied for evaluation cytotoxic effects of nanoselenium on macrophages and were compared with control cultures. This study was carried out according to Ethical Committee on Research of medical sciences faculty of Tarbiat Modares University.

*Intracellular Amastigote assay:*

After extraction of peritoneal cavity macrophages of BALB/c were seeded in 24 wells plates and incubated at 37$^{\circ}$C with 5% $CO_2$ for 24 hours for differentiation of macrophages. These cells were infected with promastigotes in stationary growth phase at a parasite/ macrophage ratio of 10:1. Durg susceptibilities of intracellular amastigotes were done with a brief modification that previously described [30]. This mixture was incubated at 37$^{\circ}$C in the presence of 5% $CO_2$ for 24 hours until promastigotes were phagocyte by macrophages. After each well of the plate was washed with 1-2 mL PBS to remove the extracellular promastigotes. Then infected macrophages were separated from the plate by cold methods [10-15 minutes on ice pieces]. At the beginning 5 $\mu$l of these cells were stained by Giemsa method. The percentage of infected cells and the number of amastigotes in each cell was microscopically assessed. Then 100$\mu$l of these cells were transferred in to new plate and were incubated with seven dilutions of selenium nanoparticles at 37$^{\circ}$C with 5% $CO_2$for 24,48,72 hours. Finally, the plates were incubated on ice pieces for 10-15 minutes. The percentage of infection and $IC_{50}$ was calculated through examination of 200 macrophages and the number of amastigotes in every single cell was determined. The results were expressed as the infection index, which is reflecting of drug effect in prevention of infection.

*Promastigote Apoptosis:*

At first $2\times10^6$ cells/ml of Promastigotes were treated with various dilutions [10, 25, 50, 100 $\mu$l/ml] of selenium nanoparticles in ELISA plate and incubated at 24$^{\circ}$C for 72 hours. Test and control wells were washed twice by cold PBS solution and centrifuged in 1400×g for 10 min. 100 $\mu$l Annexin-V FITC solution and 100 $\mu$l PI [Propidium Iodid] solution were added and incubated for 15 minutes at room temperature. Subsequently, Cellular apoptosis in our study was detected by using Annexin-V FLUOS staining kit [Roche, Germany]. The procedure was performed according to manufacturing protocol in the dark place and was evaluated FACS Calibor system. Afterwards the flowcytometery results were then analyzed using Cellquest software on the basis of 4 areas: the cells staining with Annecin-V only as apoptotic cells [LR], the cells stainin with PI as necrotic cells [UL], the cells staining with both of Annecin-V and PI as late apoptotic [UR] and those cells that did not stain as healty cells [LL].

*In vivo study:*

In this study used of BALB/c male mice with same age and the average weight of 18-20g were used. The mice were randomly sorted into four groups: two test groups contain 15 mice as treated with 5mg/kg of nanoselenium and 15 infected mice as treated with 10mg/kg, 15 infected mice as positive control group that kept without any trdatment during this study and finally 10 unifected mice for negative control group. At first 0.5ml of promastigotes [$2\times10^7$ cell/ml] was intravenously inoculated to cause infection. The infected mice were kept about 3 weeks for the establishment of Visceral leishmaniasis. Before treatment initiation the presence of infection was assessed inthree mice by culture of splenic aspirate and smears and amastigotes were counted in 10 fields with high magnification [×1000]. Drug treatments with nanoselenium were started immieiately after infections at dosages 5 or 10mg/kg of body weight. Test groups were treated daily for 3 weeks. During the treatments period [4, 10, 15, 20 days] and 3 weeks [day 40] after treatment [for insurance of recurrence disease], all mice groups were sacrificed. Spleen and liver from individual of mice were removed and isolated under sterile conditions. These organs were transferred to petri dishes containing sterile cold PBS. Then they were gently smashed to pieces by pressing the plunger of syringe to aspirate suspension. The impression smears were prepared from livers and spleens of each mouse and stained by Giemsa method and compared with control groups. Direct counting was applied to determine the percentage of infected cells and also the number of amastigotes/100 macrophage cell. Amastigote burden was compared for both test and control groups. All procedures for human care of animal were approved by the Ethical Committee on Research of medical scinces faculty of Tarbiat Medares University.

*Cytokine assay [IL-4 and INF-$\gamma$]:*

Lymphocyte were removed from spleen in test and control groups. The mice should be handled carefully but decisively to avoid stressing the animal. The mice spleens were clean to remove fatty connective tissue and then were transferred in to small petri dishes containing 5 ml of sterile cold PBS. Gently press the plunger of a syringe repeatedly on the pieces of spleens and then aspirate the suspensions and deposit it in a tube. These suspensions were centrifuged in 300×g at 4$^{\circ}$C for 10 minutes. Supernatant was discharged and then 5ml RBC lyses buffer [NH4Cl] was added to pellet and prepared suspension was incubated at room temperature for 6 minutes. After incubated 5 ml sterile cold PBS contained 2% FCS and EDTA [5Mm] was added to this suspension and centrifuged in 1500×g at 4$^{\circ}$C for 10 minutes. In next step supernatant was discharged slowly and the resultant pellet was re- suspended in 5ml cold PBS and incubated in room temperature for 10 minutes. 3ml of supernatant was transferred in to new tubes containing 5ml RPMI supplemented with 2% FCS. Direct counting after staining by trypan blue was employed for evaluating the number of live lymphocytes [approximately $1\times10^7$ cell/ml]. These suspensions were centrifuged in 300×g at 4$^{\circ}$C for 10 minutes and supernatant was discharged after that 2ml of RPMI that supplemented with 10% FCS was added to pellet. 500 $\mu$l Of lymphocytes solution from every mice separately was added to 24 wells plate contented 500 $\mu$l RPMI with 10% FCS. Then, 50 $\mu$l of SLA [soluble Leishmania antigen[ was added to each well. These plates were incubated at 37$^{\circ}$C with 5% CO2 for 48 and 72 hours. After incubation time the supernatants were collected gently and then divided in to 500 $\mu$l tubes. These tubes were frozen in -70$^{\circ}$C. Finally for the evaluation of INF-$\gamma$ and IL-4 cytokines in test and control groups DuoSet ELISA Development INF-$\gamma$ and IL-4 kits [R&D] was used. This procedure was performed according to manufacturing protocols and analyzed by ELISA system. Results in test mice were compared with control mice and were analyzed with Mann-Whitney, Post Hoc and ANOVA statistic tests.

*Statistical analysis:*

These data were analyzed by Gaph pad Prism version 5.04 software

*Results:*

Results in our study shows the effectiveness of nanoselenium on proliferation of promastigotes forms. The cytotoxic effects for seven dilutions of nanoselenium on promastigotes was obserned and compared with control cultures by a light microscope. 50% inhibitory concentration of Nanoselenium on promastigotes [$IC_{50}$] was

generated using the direct counting method [$IC_{50}$=25 $\mu$g/ml]. The growth curves of promastigotes are ahown in Figure 1.

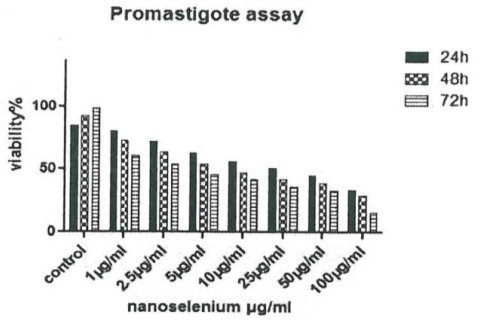


**Fig. 1:** Viability of promastigote in presence seven dilutions of nanoselenium was compared in the test and control groups in 24 and 27 hours.

MTT method was used for verification results of promastigote assay. Cytotoxicity and viability results were obtained by were showed in Figure 2.

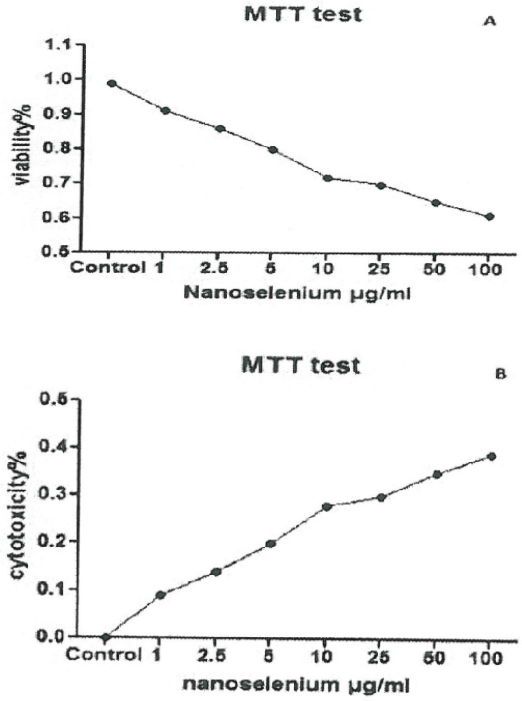


**Fig. 2:** Evaluation effects of nanoselenium in our study on promastigote of Leishmania infantum by MTT method and result were compared with control group. Figure A: viability of promastigotes in presence seven dilution of nanoselenium at 72 hours and Fig B: Cytotoxicity and seven dilution of nanoselenium on promastigotes at 72 hours.

Cytotoxicity effect of seven solutions of and nanoselenium on uninfected splenic mocropages of BALB/c mouse was compared with control cultures at 24, 48, 72 hours. These results were showed as Figure 3. And was reveales these nanoparticles had low cytotoxicity effect on uninfected promastigotes.

In this survey was assayed nanoselenium against amastigotes of Leishmania infantum and in vitro conditions was simulated like as natural body. Direct counting results were compared with control groups. 50% inhibitory concentration of Nanoselenium [$IC_{50}$] on amastigotes was calculated [IC50=10$\mu$g/ml]. These results are shown in Fig4.

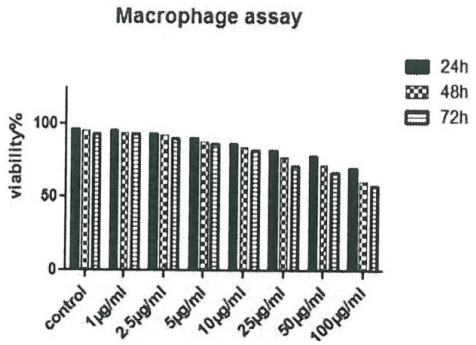


**Fig. 3:** Viability of uninfected mouse macrophages in presence of seven dilutions of nanoselenium in vitro conditions was compared whit control group at 24,48, 72 hours.

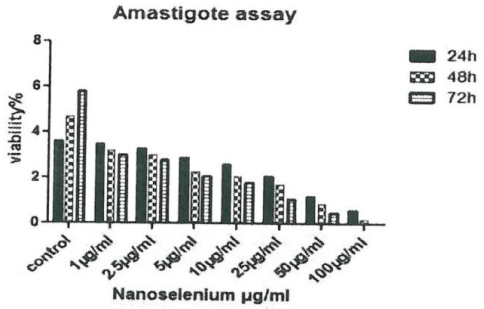


**Fig. 4:** viability for infected mouse macrophages with amastigotes of Leishmania infantum in presence of seven dilutions of nanoselenium in vitro conditions was compared in test and control group at 24,48,72 hour.

Induction of apoprosis in promastigotes of Leishmania infantum was analyzed by flow cytometer FACSCalibor system after staining with Annexin-V and PI. Then results were analyzed by Cellquest software. The percentages of apoptotic, necrotic and viable cells were determined : UL as necrotic cells in upper left region, UR as cells were banded with Annexin-V and PI in Upper right region, LL as viable promastigotes in Lower left region and LR as a marker of apoptosis in Lower right region region of figures. From these results can be concluding Nanoselenium were induced apoptosis in promastigote cells at 5 concentrations. Results are shown in Fig 5.

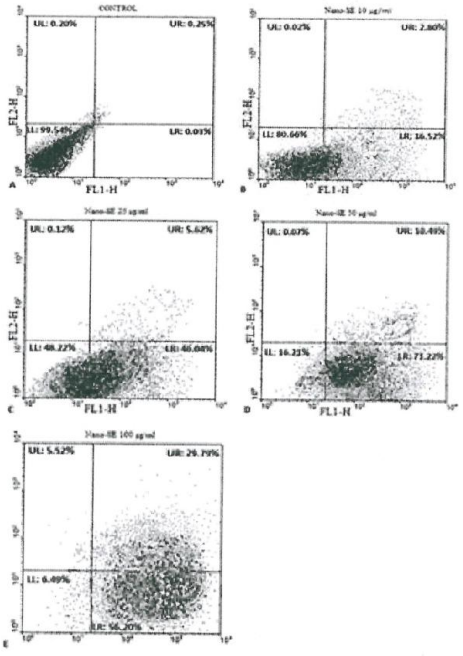


**Fig. 5:** Induction of apotosis in promastigotes of Leishmania infantum evaluated by flow cytometery method.

Figure A are shown the most of promastigotes were alive and healthy as control group, B as 10$\mu$g/ml, C as 25 $\mu$g/ml, D as 50 $\mu$g/ml and E as 100 $\mu$g/ml.

In this study for determination effects of Nanoselenium on visceral Leishmaniasis in vivo conditions used two concentrations of Nanoselenium with 40 nm sizes results were obtained by presence of infected macrophages and amastiotes inside spleen of each mouse in 4,10,15,20,40 days after treatment with nanoparticles. Mean of amastigotes/100 macrophage of spleen cells, standard deviation and standard error were compared with control groups by ANOVA statistical test. The mean of amastigote within macrophages in 10 fields with high magnification [×1000] were decreased and were compared with control groups. Interestingly the difference was considered statistically significant among two test [5mg/kg and 10mg/kg] and control groups [P≤0.006]No satistically difference was found between 5mg/kg and 10mg/kg of Nano selenium concentrations in amastigote reduction by [P≤0.006]. Relapse infectons in tests groups compared with control groups, were decreased significantly in 3 weeks after the end of treatment course. The mortality rates in test groups compared to all control groups were decreased significantly. Low mortality rate observed in test groups and high mortality was seen in control group without any treatment [Table 1].

Evaluation of INE-$\gamma$ and IL-4 cytokines levels in test and control groups were analyzed by ELISA reader system at 24 and 48,72 hours after culture. Results of 5 and 10 INF-$\gamma$ and IL-4 standard curves and control groups. These results are presented in Figs6.

The Mann Whitney, Post Hoc and ANOVA statistical tests were employed for multiple comparisons and analysis of IL-4 and INF-$\gamma$ cytokines results. These results were showed that nanoselenium was increased INF-$\gamma$ and IL-4 cytokine levels in treated mice rather than control groups [Figure.

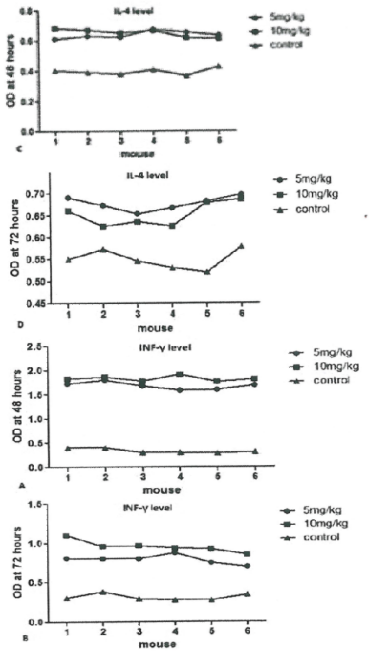


**Fig. 6:** Evaluation of INF-$\gamma$ and IL-4 cytokines levels in test and control groups were analyzed by ELISA reader system.

**Table 1:**

| mean±SD/Se | Control | 5mg/kg | 10mg/kg |
|---|---|---|---|
| INF-$\gamma$[48] | 0.332±0.05 | 1.683±0.07 | 1.826±0.05 |
| INF-$\gamma$[72] | 0.315±0.04 | 0.790±0.06 | 0.960±0.08 |
| IL-4 [48] | 0.397±0.02 | 0.639±0.02 | 0.651±0.02 |
| IL-4 [72] | 0.550±0.02 | 0.677±0.01 | 0.652±0.02 |

**Table 2:** Results [Mean ±standard deviation/ standard error] of INF-$\gamma$ and IL-4 levels were determined at 48 and 72 hours by using ANOVA statistical test [p≤0.05].

| Variable | Group 5/10 | Group 5/control | Group 10/control | ANO VA |
|---|---|---|---|---|
| IL-4[48] | 0.646 | 0.000* | 0.000* | 0.000* |
| IL-4[72] | 0.821 | 0.029 | 0.010* | 0.006* |
| INF-$\gamma$[48] | 0.193 | 0.000* | 0.000* | 0.000* |
| INF-$\gamma$[72] | 0.001* | 0.000* | 0.000* | 0.000* |
| | | | | |

P-value for cytokine responses [INF-$\gamma$ and IL-4] of lymphocytes were compared between infected mice after treatment with 5 and 10 mg/kg concentrations of nanoselenium and control groups by Post Hoc and ANOVA tests for multiple comparison at 48 and 72 hours [Table3].

**Table 3:** P-value obtained from comparison of INF-$\gamma$ and IL-4 levels between test and control groups. Data was analyzed by Post Hoc and ANOVA test.

| Mann- Whitney Test [P-value] | IL-4[48] | IL- 4[72] | INF-$\gamma$[48] | INF-$\gamma$[72] |
|---|---|---|---|---|
| 5/10mg/kg | 0.522 | 0.749 | 0.150 | 0.006* |
| Control/5 mg/kg | 0.006* | 0.018* | 0.006* | 0.006* |
| Control/10mg/kg | 0.006* | 0.006* | 0.006* | 0.006* |

The Mann-Whitney test was used for comparison of INF-$\gamma$ and IL-4 responses and P-value between test and control mice at 48 and 72 hours [Table4].

Statistical analysis for cytokine assay was performed by using SPSS statistical

*Discussion:*

Nano medicine is a reality that is producing advances in the diagnosis, prevention and treatment of disease. In present study we attempt evaluate the effects of selenium red nanoparticles synthesized by biological method on Leishmania infantum and visceral leishmaniasis. Therefore we simulated in vitro and in vivo vonditions like as normal cycle of Leishmania. Red nanoselenium is identified as a potent antileishmania agent against promastigotes and amastigotes but in contrast it showed lower toxicity toward uninfected macrophages. Programmed cell death [apoptosis] was analyzed by flow cytometer and showed that selenium nanoparticles were induced apoptotic effects on promastigotes of Leishmania infantum [MHOM/TN /80/IPI1]. Cells apoptosis can be very effective in elimination of parasite. Nonspecific and specific immune systems are intended to elimination of the foreign agent. During the leishmaniasis, the cells produce various cytokines, such as tumor necrosis factor [TNF] and interferon [IFN] what that facilitate activation of macrophages and the development of Th1 response in the mouse model [31,32]. Interferon is a glycoprotein secretion released by many cell types in responses against infection [33]. The specific mechanisms are including the cellular and humeral inmmune responses [34].

Before reinforcements arrive, macrophages and other immune cells located in the infection area and begin to phagocyte, cut into pieces called antigens. Monocytes are main cells against to Leishmania infection. Macrophages have various roles in the immune response such as: antigen recognition, antigen presenting to T lymphocyte, phagocytosis and etc. [35,36,37, and 38]. The number of cells such as monocytes [macrophages], neutrophils and eosinophil have mechanisms are useful to control of diseases for example against enzymes and oxidative radicals, but the parasite manages to evade most of these host defense mechanisms and these defenders molecules may also be involved in the development of clinical manifestations of leishmaniasis [39]. Unfortunately, Leishmania can interfere with these requirements and prevent the subsequent development of a protective response[40]. In peripheral blood mononuclear cells [PBMC] from experimentally infected dogs, demonstrated an association between a response Th1 and resistance to VL. In the presence of a pathogen, the chemokine can activate T lymphocytes and macrophages, and recruit appropriate effector cells to the infection area in the spleen of experimentally infected dogs by L. donovani [41,42,43,44,45]. In present study was demonstrated the ability lymphocytes of infected mice for produce interferon gamma during treatment by nanoselenium. This study was revealed a significant IFN- response in the spleen cell cultures of test groups compared with control group. IFN- is an important cytokine that is critical for immune cellular response against Leishmania. The importance of IFN- is immune stimulatory, immune regulatory and modulatory effects. IFN- is produced by [NK] as part of the innate immune response and by T helper cells and cytotoxic T cells [46,47,48]. IFN- is a promotive factor for macrophage activity, antigen presentation and lysosome inducible

Nitric Oxide Synthase activity of macrophages. In leishmaniasis disease IFN- promotes Th1 differentiation and macrophage activity [49]. The mechanism of action of Nanoselenium against Leishmania is unknown. By in vivo experiments we have found that amastigotes of Leishmania infantum are very susceptible to selenium nanoparticles. Nanoselenium could have decreased the amastigote rate in spleen and liver of mice during the treatment. This study has shown that selenium nanparticles can be used for treatment strategies against leishmaniasis. In summary, these satisfactorily results demonstrated that nanoselenium can be a potential candidate for further evaluation as a chemotherapeutic agent for the treatment of lishmaniasis. Further study on larger groups of animals and different concentrations of the solutions are required. This finding brings us more interesting in studying these Nanoparticles. Nanoselenium had remarkable therapeutic effect on visceral leishmaniasis and was decreased progression of this disease in BALB/c mouse model, now can say nanoselenium is valuable in the future medical studies in leishmaniasis treatment.

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