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Original Article

Evaluation of Anti-leishmanial Effect of Selenium Nanoparticles on *Leishmania major* Promastigotes *In Vitro*

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Abstract

Background: *Cutaneous leishmaniasis* (CL) remains a *major health* threatening disease in Iran and many countries around the world. Antimony compounds are currently used to treat CL. Due to the side effects and high resistance, the use of alternative therapies, especially the use of nanoparticles, has been considered by researchers. The aim of this study was to investigate the anti-leishmanial activity of selenium *nanoparticles* (SeNPs) on *Leishmania major in vitro*.

Methods: In this experimental study, the anti-leishmanial activity of the SeNPs was evaluated at concentrations of 1.25, 2.5, 5, 10, 25, 50, and 100 µg/mL at exposure times of 24, 48, and 72 hours on 10^6 live parasites. Then, the number of live parasites was counted by trypan blue using a neobar slide and light microscope (Hemocytometer method). Glucantime and distilled water were considered positive and negative controls, respectively. Then, 50% inhibitory concentration (IC50) values were calculated by SigmaPlot™ 13 software. All reactions were performed in triplicate, and the results were considered as average.

Results: The results of this study revealed that all concentrations of SeNPs have anti-leishmanial activity. The concentration of 100 µg/mL of SeNPs had the highest anti-leishmanial effect (100%) after 72 hours of exposure. Further, the IC50 content of SeNPs on *L. major* after 24, 48, and 72 hours was calculated to be 42.76, 34.53, and 22.69 µg/mL, respectively.

Conclusions: The results indicated that SeNPs in different concentrations has an inhibitory effect on the growth of *L. major*. However, further investigations are required to determine the efficacy of SeNPs *in vivo*.

Keywords: Selenium *nanoparticles*, Anti-leishmanial activity, *Leishmania major*, *In vitro*

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Introduction

Leishmania strains cause a range of human diseases in tropical and subtropical regions around the world (1), which are manifested as cutaneous leishmaniasis (CL), muco-cutaneous leishmaniasis, and visceral leishmaniasis (2). The disease is caused by an obligatory intracellular protozoan called *Leishmania* of the order Kinetoplastida. Approximately 350 million people worldwide are at risk for the disease, and 2 million new cases are reported annually (3,4). Antimony compounds have been at the forefront for the treatment of this disease for decades, but these drugs have toxic properties on the liver, heart, and kidneys, require repeated injections, and cause resistance to the parasite (5-7). Therefore, it seems necessary to provide a more effective drug with fewer side effects and faster wound healing. Accordingly, researchers have turned to the use of nanoparticles to treat infectious diseases in recent years. Nanotechnology is currently being proposed as a new way to combat parasites and their diseases (8,9). In recent years, some nanoparticles (e.g., gold, selenium, silver, and sulfide) have been used to treat leishmaniasis.

According to some studies, selenium nanoparticles (SeNPs) are more effective in treating diseases than selenium itself. SeNPs with antioxidant and equally pro-oxidant properties provide different ways to explore different pathological conditions (10). SeNPs have different applications including acting as an antioxidant, improving learning, enhancing hair growth being anti-bacterial and anti-cancer aiding digestion, modulating the immune system, and improving reproductive and growth

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functions (11). Due to the different properties of these nanoparticles, this study was carried out to investigate the anti-leishmanial activity of SeNPs on *Leishmania major* parasite in vitro.

Materials and Methods

Parasite Culture

The standard strain of *L. major* promastigote (MRHO/IR/75/ER) was prepared from Pasteur Institute of Iran and cultured in *Novy*–MacNeal–*Nicolle* two-phase culture medium. Then, it was transferred to RPMI-1640 culture medium with antibiotics, including penicillin (100 units/mL), streptomycin (100 µg/mL), and 20% fetal bovine serum for mass propagation. Afterwards, flasks of culture medium were incubated at 25 ± 1°C and examined daily by reverse microscopy (invert).

Characterization of SeNPs

SeNPs nanopowder was purchased from Pishgaman Iran Nanomaterials Company. The average particle size of SeNPs was 30 nm, true density was 3.89 g/cm^3, specific surface area was 30-50 m^2/g, and purity was 99.95%. Its properties were determined by scanning electron microscopy (SEM, EM3200, KYKY Technology Development Ltd, Beijing, China) and transmission electron microscopy (TEM, Leo 906, Zeiss 100 KV, Germany). Concentrations of 1.25, 2.5, 5, 10, 25, 50, and 100 µg/mL were prepared from SeNPs by dissolving it in distilled water. The diluted extracts were sterilized by a 0.22 µm syringe filter with a diameter of 25 mm made by Sartreus, Germany.

Challenging the SeNPs With Leishmania Parasite and Determining the IC50

To investigate the lethal effect of SeNPs on promastigotes of *L. major* parasite, 10^6 parasites in logarithmic phase were exposed to concentrations of 1.25, 2.5, 5, 10, 25, 50, and 100 µg/mL. Then, 40 µg/mL glucantime and distilled water were used as positive and negative controls, respectively. Three flasks were considered for each concentration as well as for positive and negative controls. All groups were incubated at 25 ± 1°C, and after 24, 48, and 72 hours, the number of live parasites was counted by Trypan Blue using a neobar slide and light microscopy (Hemocytometer). The mortality rate for each of the different concentrations of SeNPs on the parasitic promastigotes was calculated by the following formula:

GI = (a-b / a)

where a is the number of live parasites in the negative control sample, and b is regarded as the number of live parasites in the sample containing SeNPs. Then, the amount of 50% inhibitory concentration (IC50) for the above extract was calculated using SigmaPlot™ 13 software.

Statistical Analyses

In this study, all data were entered into Excel software to report the results as mean and standard deviation, and SigmaPlot™ 13 software (Systat Software Inc., San Jose, CA, USA) was used to calculate IC50.

Results

The effect of different concentrations of SeNPs (1.25, 2.5, 5, 10, 25, 50, and 100 µg/mL) after 24, 48, and 72 hours of exposure to *L. major* promastigotes were studied in vitro. The IC50 content of SeNPs after 24, 48, and 72 hours on *L. major* was calculated to be 42.76, 34.53, and 22.69 µg/mL, respectively. The highest anti-leishmanial effect (100%) was observed at a concentration of 100 µg/mL after 72 hours of exposure. Table 1 presents the amount of *Leishmania* promastigotes being exposed to different concentrations of SeNPs after 24, 48, and 72 hours of incubation, and Table 2 illustrates the rate of inhibition of *Leishmania* promastigotes. Moreover, Figure 1 displays the average lethal percentage of SeNPs on *L. major* promastigotes, and Figures 2 and 3 represent the TEM and SEM images of SeNPs, respectively.

Discussion

According to the World Health Organization (WHO), approximately 14 million people in Africa, Asia, Europe, and the US are directly affected by leishmaniasis (12). Antimony is recommended as the main drug to treat leishmaniasis, but there are limitations such as high costs, side effects, repeated injections, and ineffectiveness; therefore, the preparation of a new anti-leishmanial drug

Table 1. Number of *Leishmania* Promastigotes Against Different Concentrations of SeNPs After 24, 48, and 72 Hours of Incubation

Concentration(µg/mL)	24 Hours	48 Hours	72 Hours
1.25	2182	3554	5086
2.5	2088	3350	4596
5	1900	3064	4105
10	1759	2819	3247
25	1525	2206	2818
50	1102	1593	1715
100	187	123	0
Positive control	0	0	0
Negative control	2346	4086	6128

Note. SeNPs: Selenium *nanoparticles*.

Table 2. The Effect of Different Concentrations of SeNPs on Promastigotes of *Leishmania major* Parasites After 24, 48 and 72 Hours of Incubation

Concentration(µg/mL)	24 Hours (%)	48 Hours (%)	72 Hours (%)
1.25	7	13	17
2.5	11	18	25
5	19	25	33
10	25	31	47
25	35	46	54
50	53	61	72
100	92	97	100
Positive control	100	100	100
Negative control	0	0	0

Note. SeNPs: selenium *nanoparticles*.

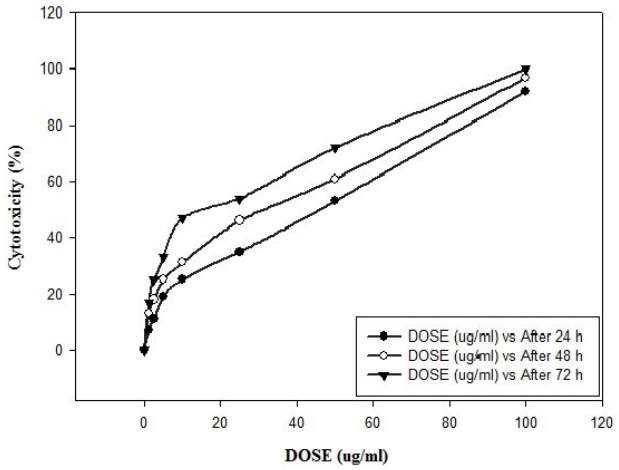

Figure 1. Mean Mortality of SeNPs on Promastigotes of *Leishmania major*. *Note.* SeNPs: Selenium *nanoparticles*

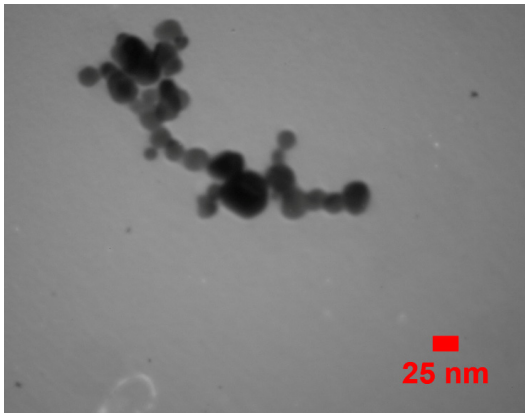

Figure 2. TEM Image of SeNPs in 25 nm. *Note.* TEM: Transmission electronic microscopy; SeNPs: Selenium *nanoparticles*

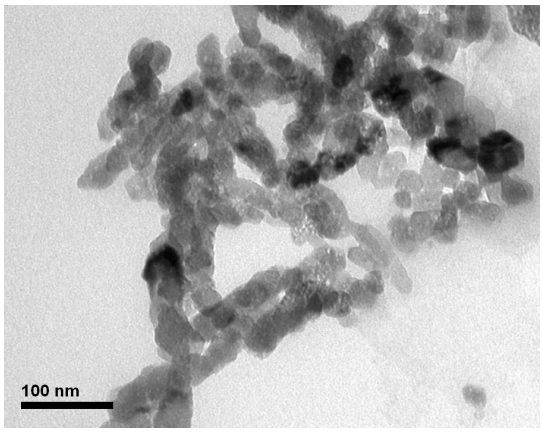

Figure 3. SEM Image of SeNPs in 100 nm. *Note.* SEM: Scanning electronic microscopy; SeNPs: Selenium *nanoparticles*.

is one of the most essential needs. Therefore, the aim of this study was to investigate the anti-leishmanial activity of SeNPs on *L. major in vitro*. The highest lethality (100%) was observed at a concentration of 100 µm/mL after 72 hours of exposure. One of the mechanisms by which selenium exerts its beneficial effects on the health is through selenoprotein. In addition, selenium replaces sulfur in methionine in the form of selenomethionine, which can be incorporated into non-specific proteins (13).

Mayelifar et al incubated *L. major* promastigotes with silver NPs for 1 hour and then applied 8 electrical pulses with an electroporation device. The results showed that the presence of silver NPs and the simultaneous application of electrical pulses lead to a significant decrease in parasite survival (14). Akhzari et al used activated melittin along with liposomes and albumin NPs to treat leishmaniasis wounds caused by *L. major*, and they found that the size of wounds in this group significantly reduced (15). Further, a study by Casa et al revealed that albumin NPs containing amphotericin B reduced the toxicity of the drug and reduced the tissue toxicity of the drug to the tissues of the liver, spleen, heart, and kidney as well (16). In another study, Haddad et al reviewed the anti-leishmaniasis effect of curcumin-laden chitosan NPs on *L. major* and *Leishmania infantum* under in vitro conditions, and counting the number of parasites indicated that the synthesized NPs have a favorable effect in growth inhibition (17). The results of another study by Torabi et al demonstrated that gold NPs reduce the number of *L. major* amastigotes in Leishmaniasis wounds and reduce the mortality rate of mice (18).

The effects of SeNPs on *L. infantum* were investigated by Soflaei et al, and IC50 NPs against amastigotes and promastigotes of *L. infantum* were reported to be 10 and 25 µg/mL, respectively (19). Excretory-secretory *L. major* on macrophage apoptosis represented that chitosan can increase the ability of infected macrophages to remove parasites by reducing apoptosis (20). Sazgarnia et al surveyed the effect of gold NPs and microwave radiation on the survival of *L. major* promastigotes and amastigotes, concluding that the presence of gold NPs during microwave irradiation is more lethal to promastigotes and amastigotes; therefore, it could be suggested as a new approach for treating leishmaniasis (21). In another study, Jameii et al (22) examined the anti-leishmaniasis effect of selenium and silver NPs on *L. major* wound healing and found that the diameter of nano-selenium group wounds does not show a significant difference with the control group, while the diameter of nano-silver group wounds exhibited a significant difference with the control group, which is larger than the group treated with glucantime (positive control).

In the study by Baiocco et al which evaluated the lethal effect of silver NPs against visceral leishmaniasis, the results displayed that, compared to antimony compounds, silver NPs have a greater effect on *Leishmania* mortality (23). In a review study, Elmi et al exhibited that NPs of silver, gold, chitosan, and metal oxides have a lethal or inhibitory effect on *Leishmania* (24). In another study, Jebali and Kazemi examined the effects of NPs of silver, gold, titanium dioxide, zinc dioxide, and magnesium dioxide under ultraviolet light, infrared, and dark conditions, observing the highest anti-leishmanial activity for silver NPs; further, both ultraviolet and infrared light were found

to have anti-leishmaniasis activity (25). El-Khadragy et al biosynthesized silver NPs using *Moringa oleifera* leaf extract and investigated the anti-leishmaniasis activity of these NPs in a mouse model with *L. major* infection. They concluded that treatment with biosynthesized silver NPs using *M. oleifera* extract have a higher and faster clinical effect than treatment with standard pentavalent antimony possibly due to increased antioxidant activity (26). Different results obtained from various studies are due to different NPs and the differences in measurement units such as micrograms, milligrams, and the like.

The results of this study indicated that all concentrations of SeNPs have anti-leishmanial activity, and a concentration of 100 µg/mL of SeNPs has the highest anti-leishmanial effect (100%) at 72 hours of exposure. However, further in vivo conditions are required to determine the performance of SeNPs.

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Conflict of Interests

The authors declared no conflict of interests.

Ethical Approval

This study was approved by the research committee of Qom University of Medical Sciences (IR.MUQ.REC.1400.185).

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