Anti-leishmania Effect of Magnesium Oxide Nanoparticles on *Leishmania tropica*/*infantum* and *Leishmania*-Infected Macrophages

Amir Karimipour-Saryazdi1,*, Mohammad Mahdi Jafari2, Roya Omidi1, Fatemeh Ghaffarifar1, Seyyed Hojjat Sadeghi3

1Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3Organic and Nano Group (ONG), Department of Chemistry, Iran University of Science and Technology (IUST), Tehran 16846-13114, Iran

Abstract

**Background:** *Leishmania* is an intracellular protozoan parasite that enters and reproduces in macrophage cells. Macrophages are important immune cells that phagocytose many pathogens such as bacteria, fungi, and parasites such as *Leishmania* spp. but are incapable of killing this parasite, living in the phagosomes of infected macrophages, multiplying, and resulting in the devestating of infected macrophages and the appearance of *Leishmania* lesions. Many of the present drugs for *Leishmania* treatment have side effects, or parasites have resistance to some of these drugs. Therefore, there is a need for a better drug for *Leishmania* treatment. Magnesium oxide (MgO) is a metal nanoparticle (NP) with numerous biological applications, including antioxidant and antimicrobial effects on various pathogens such as some bacteria, fungi, and parasites, including *Leishmania* spp.

**Objectives:** Accordingly, this article has discussed the effects of MgO NPs on *Leishmania tropica* and *Leishmania infantum* and *Leishmania*-infected macrophages.

**Materials and Methods:** The effect of various doses of MgO NPs on *L. tropica* and *L. infantum* promastigotes and amastigotes was studied in vitro. Flow cytometry and MTT were also utilized to assess the cytotoxic effects of MgO on *L. tropica* and *L. infantum* promastigotes, as well as the likelihood of apoptosis. Amastigote assay was employed to determine the infected macrophage percentage, and the number of parasites present in every macrophage cell. A percentage of macrophages contaminated with amastigotes of *L. tropica* and *L. infantum* that were treated with MgO NPs was 15% and 11%, respectively. Flow cytometry revealed that MgO NPs induced approximately 38.56% and 30.5% apoptosis on *L. tropica* and *L. infantum*, respectively. The half maximal inhibitory concentration of MgO NPs to *L. tropica* and *L. infantum* according to promastigote assay for 72 hours was 7.32 μg/mL and 12.58 μg/mL, respectively.

**Conclusion:** According to the findings, MgO NPs had a great in-vitro fatality effect on *L. tropica* and *L. infantum* promastigotes and amastigotes (inside *leishmania*-infected macrophages).

**Keywords:** Magnesium oxide, *Leishmania tropica*, *Leishmania infantum*, *Leishmania*-infected macrophages

**Background**

Leishmaniasis is a tropical and subtropical serious infection of the genus *Leishmania* and is spread via the bite of an infected female *phlebotomine* sandfly.1,2 This disease is one of the most important global health issues, and the poorest continents in the world are most vulnerable to *Leishmania* parasite morbidity and mortality. At least three different forms of leishmaniasis have been described, including cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL).3,4 Infection with *Leishmania chagasi* creates the VL disease (also known as kala-azar), which is primarily found in the Mediterranean basin, Central Asia, and Brazil, and has recently spread to major Brazilian cities. VL is the most dangerous form of leishmaniasis that is often deadly if ignored.3

Except for a few human cases of clinical VL, the majority of infections are asymptomatic.4,5 VL symptoms include swelling of the spleen, sporadic fever, and loss of appetite.4

The most dominant reservoirs of *L. infantum* are dogs,5 and the parasite is transmitted by the female *Phlebotomus perniciosus* sand fly.6 *Leishmania tropica* is one of the parasites that causes CL, a disrupting beauty...
disease that has been identified as viscerotropic. In urban areas, phlebotomine sand flies (*Phlebotomus sergenti*) bite uninfected people to spread the disease. In non-urban areas, animals are thought to be the parasite’s reservoir.\(^{15}\) *L. tropica* is widely spread in Syria, India, Iraq, the Greek islands, Turkmenistan, Algeria, Afghanistan, and other tropical areas.\(^{9}\) Some medications or chemical agents such as sodium stibogluconate, miltefosine, and paromomycin are used to treat VL,\(^{7}\) while others such as sodium stibogluconate (Pentostam), meglumine antimonate (glucantime), paromomycin, pentamidine, or amphotericin B currently are prescribed to treat CL.\(^{15}\)

Macrophages are among the most important immune cells that have two phenotypes (M1 and M2).

The M1 phenotype has an inflammatory effect that produces some pro-inflammatory mediators such as tumor necrosis factor α, as well as interleukin 1 (IL-1), IL-6,\(^{12}\) and some metabolic products such as reactive oxygen species (ROS), along with nitric oxide (NO). All of these products, after digesting microbes (phagocytosis) and then fusion phagosomes with the lysosome, kill microorganisms.\(^{13}\) Some products, including IL-4, IL-10, tumor growth factor B, and trophic polyamines, inhibit inflammation and the formation of phagolysosomes.\(^{12,13}\)

Some pathogens such as *Leishmania* survive in phagosomes and multiply, killing macrophages and causing injury or histopathology by inhibiting phagolysosome formation, inflammatory cytokine production, and arginase (NO) production\(^{14,19}\) and inducing the M2 phenotype.\(^{12,19}\)

Studies show several organic nanoparticle (NP)-based substances have anti-leishmanial effects.\(^{20-22}\) Metal NPs are compounds that have significant applications in the field of medicine, including leishmaniasis.\(^{23,24}\) Selenium, gold, silver (Ag), zinc oxide, titanium dioxide, and magnesium oxide (MgO) are the most important metal NPs that have anti-Leishmanial effects.\(^{22,25-28}\) MgO is one of the metal NPs with a wide range of biological applications, including antioxidant and antimicrobial effects\(^{29,29,32}\) on various pathogens such as some fungi\(^{33}\) and bacteria,\(^{34,38}\) as well as parasites such as *Leishmania* spp.\(^{36-38}\) Current leishmaniasis treatments are costly, toxic, have a wide range of side effects,\(^{3}\) injections are painful, and courses of treatment are long. Drugs are scarce, and this parasite has resistance to some of these drugs.\(^{3,4,11,22,43,44}\) On the other hand, infected macrophages with amastigotes have long been the reservoirs of *Leishmania*.\(^{45}\) Hence, using some compounds such as NPs, including MgO and other NPs, is an appropriate choice for the treatment of leishmaniasis because these compounds are available. They are also somewhat cheap and have no or little side effects.\(^{46-48}\) Furthermore, these compounds induce apoptosis in macrophages or clean the parasites from inside macrophages and remove the reservoir of parasites.\(^{25,49,50}\)

The purpose of this study is to find how MgO NPs affect *L. tropica/infantum*, and *Leishmania*-contaminated macrophage cells.

## Materials and Methods

### Magnesium Nanoparticles’ Synthesis

The Department of Pharmaceutical Biotechnology Research and the Research Center for Pharmaceutical Sciences, Faculty of Pharmacy, Tarbiat Modares University, Tehran created MgO NPs. Various NP concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 μg/mL) were prepared for the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and promastigote assay.

### 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium Bromide Assay for Macrophage

A murine macrophage cell line (RAW 264.7) was utilized for the MTT assay in this study. In a dark room, 5 mg of MTT powder (Sigma Chemical Company, Germany) and 1 mL of PBS were mixed to create an MTT solution. The cells were grown in Dulbecco’s Modified Eagle medium that had been enhanced with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37°C with 5% CO\(_2\). The cells (5 × 10\(^{4}\) /mL) were seeded into a 96-well plate, and various concentrations of MgO NPs (400, 200, 100, 50, 25, 12.5, and 6.25 μg/mL) were added to them. The plate was incubated at 37°C for 5 hours in a dark area after adding 20 μL MTT to each well after 72 hours to permit the cells to convert the tetrazolium to an insoluble formazan. After draining the supernatant from the wells, 100 μL of DMSO was added to each well. The optical density was then assessed using an enzyme-linked immunosorbent assay (ELISA) reader (Stat Fax, USA) set to 540 nm. Finally, the following formula was used to calculate the precise cell viability rates as a percentage in the test groups and the control groups with the absorbency of the blank.

\[
\text{Viable (Live) macrophages (percentage) } = \frac{(AT-AB)}{(AC-AB)} \times 100
\]

- **AT:** Macrophage absorbance when exposed
- **AC:** Untouched macrophages absorption
- **AB:** The absorbency of the blank

### 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium Bromide Assay for L. tropica/infantum Promastigotes

MTT assay was performed to examine how MgO NPs affected *L. tropica/infantum* promastigotes. Promastigotes in the number of 5 × 10\(^3\) /mL have been cultured in 96-well plates and subjected to various concentrations of MgO NPs (400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 μg/mL). Each well received 20 μL MTT after 72 hours in a dark place. After 5 hours of incubation at 25°C, the cells were centrifuged, and the supernatant was removed. Then, each well received 100 μL of DMSO, and
Flow Cytometry Assay
A hemocytometer slide was employed to count *L. tropica*/*infantum* promastigotes (2 × 10^6), and a concentration of 40 μg/mL of MgO NPs was added to the promastigotes. After 72 hours, the cell specimens were centrifuged for 10 minutes at 1400 g. Following the addition of 500 mL of buffer, the samples were incubated for an additional 15 minutes on ice before receiving 5 mL of annexin V and 5 mL of PI (annexin-V kit, IQ Products BV, Groningen, Netherlands). The specimens were then evaluated by a flow cytometer (BD FACScanto II, USA). The device’s output is presented as graphs and percentage charts. Software called FlowJo (version 10) was used to analyze the data. This kit could distinguish between apoptotic (just annexin-V positive as primary apoptosis/lower right), both annexin-V and PI positive as secondary apoptosis/upper right), normal, living (both annexin-V and PI negative/lower left), and necrotic (PI positive/upper left) cells.\(^{11,22,52,55,56}\)

Amastigote Assay (Infected-macrophage Assay)
The 12-well plates used in this experiment were incubated for 24 hours at 37°C with 5% CO\(_2\) to allow macrophages to stick to the underside of the plates. Next, 10^5\(^{\text{m}}\) macrophages were infected with 10^5\(^{\text{m}}\) stationary-phase promastigotes of *L. tropica* and *L. infantum* (1:10) and incubated for 24 hours to create amastigotes.\(^{11,52}\) After 24 hours at 37°C with 5% CO\(_2\), the supernatant was taken out and washed with PBS to eliminate any promastigotes that did not enter the cells. Subsequently, 20 and 40 μg/mL MgO NPs were added to macrophages that had been infected, and then plates were placed in the incubator for 72 hours. The slide was removed from the plates’ bottoms, fixed with methanol, and stained with Giemsa. They were then visited with an optical microscope to evaluate the amastigote number and the macrophage percentage.

Macrophages with and without amastigotes were therefore compared in any group. In the groups of *L. tropica* and *L. infantum*, the amastigote’s mean number per macrophage was calculated and compared with the control groups (the negative control group and groups treated with glucantime [50 μg/mL]).\(^ {3,11,37}\)

Promastigote Assay
The standard strain of *L. tropica* (MHOM/IR/02/Mash10) and the World Health Organization reference strain of *L. infantum* (MHOM/TN/80/IPT1) promastigotes were cultured at 25±1°C in RPMI 1640 (Gibco, Germany) reinforced with 10% FBS (Gibco, Germany) and antibiotics (100 IU/mL penicillin and 100 μg/mL streptomycin). Every day, promastigotes in the culture were monitored to collect cells in the logarithmic phase.\(^ {52}\)

The *L. tropica* and *L. infantum* promastigotes were counted and added at a rate of 100 μL to 96-well plates that had already been loaded with 100 μL of each of the eleven different dilutions of the MgO NP (400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 g/mL). The promastigotes were evaluated with a hemocytometer slide after being exposed for 24, 48, and 72 hours at 24°C to determine their quantity and morphological characteristics, as well as compare them with control groups, including those who were not treated and those who had received glucantime (50 μg/mL) treatments. There were three copies of each experiment run.\(^ {3,11}\) Moreover, the amount of the half-maximal inhibitory concentration (IC\(_{50}\)) was determined according to the promastigote assay in this research. This amount was calculated by Quest Graph™ IC50 Calculator (AAT Bioquest,\(^ {39}\)).

Statistical Analysis
IBM SPSS (version 21) was used to conduct the statistical analyses. Data normalization was ensured using the Kolmogorov-Smirnov test, and mean differences were compared by one-way ANOVA and LSD. Finally, Graph Pad Prism (version 8.0.1.) was employed for graph drawing.\(^ {11}\)

Results
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium Bromide Assay for Macrophages
MTT assay was performed to determine the toxic effect of MgO NPs on macrophage cells. The results of the MTT revealed that the concentrations of 200 and 400 μg/mL had the most toxic effects on macrophage cells (Figure 1), while the lower doses of MgO NPs had a slight impact and did not significantly differ from the control.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium Bromide Assay for *L. tropica* and *L. infantum* Promastigotes
The MTT test was also utilized to calculate the percentage of *L. tropica* and *L. infantum* parasite viability. The outcomes of this test are depicted in Figures 2 and 3. The highest toxic effect of MgO NPs was observed at the concentration of 400 μg/mL for *L. tropica* and *L. infantum*.

Flow Cytometry Analysis
A concentration of 40 μg/mL of MgO NPs was chosen for the flow cytometry test. *L. tropica* underwent 8.56% primary and 30% secondary apoptosis in response to MgO NPs, while *L. infantum* underwent 12.2% primary and 18.3% secondary apoptosis in response to MgO NPs. In the untreated control group, 98.4% and 98% of the *L. tropica* and *L. infantum* parasite cells were alive, respectively. More comprehensive information is illustrated in Figures 4 and 5.
Figure 1. MTT Assay for Macrophage. Note. MTT: 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide; MgO: Magnesium oxide; NP: Nanoparticle. This test was performed to evaluate the viability percentage (live percentage) of macrophages in exposure to six concentrations of MgO NPs. As shown, higher MgO NP concentrations (e.g., 400 and 200 µg/mL concentrations) have the most toxic effects on macrophage cells, while lower concentrations of MgO NPs have the lowest toxic effects on macrophage cells, and these effects are ignorable compared to the control group. Thus, the toxicity effects of those on macrophages increase with an increase in MgO NPs doses.

Figure 2. MTT Assay for *Leishmania infantum*. Note. MTT: 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide; MgO: Magnesium oxide; NP: Nanoparticle. This test was conducted to investigate the viability percentage (live percentage) of *Leishmania infantum*. Based on the data, in groups that are treated with low concentrations of MgO NPs, the viability percentage (live percentage) of *Leishmania infantum* is high, while in groups that are treated with high concentrations of MgO NPs, the viability percentage (live percentage) of *Leishmania infantum* is low. As a result, as the concentration of MgO NPs increased, the viability percentage (live percentage) of *Leishmania infantum* decreased further than in the control group. In addition, at high concentrations of MgO NPs (e.g., 400 and 200 µg/mL concentrations), the viability percentage (live percentage) of *Leishmania infantum* is lower than in conventional therapies such as Glucantime.

Figure 3. MTT Assay for *Leishmania tropica*. Note. MTT: 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide; MgO: Magnesium oxide; NP: Nanoparticle. This test was performed to study the viability percentage (live percentage) of *Leishmania tropica*. As depicted, in groups that are treated with low concentrations of MgO NPs, the viability percentage (live percentage) of *Leishmania tropica* is high, whereas the viability percentage (live percentage) of *Leishmania tropica* is low in groups that are treated with high concentrations of MgO NPs. Accordingly, as the concentration of MgO NPs increased, the viability percentage (live percentage) of *Leishmania tropica* decreased further than in the control group. Further, at high concentrations of MgO NPs (e.g., 400 and 200 µg/mL concentrations), the viability percentage (live percentage) of *Leishmania tropica* is lower than in conventional therapies such as Glucantime.
Amastigote Assay
At MgO NP concentrations of 20 and 40 μg/mL, the percentages of macrophages contaminated with *L. infantum* amastigotes were 20% and 11%, respectively. However, at these concentrations, the percentage of contaminated macrophages with *L. tropica* amastigotes was 24% and 15%, respectively, and 40% of macrophages were contaminated with *L. tropica* and *L. infantum* amastigotes in the untreated control group. In addition, at a 20 μg/mL concentration, the mean number of *L. tropica* and *L. infantum* in the infected macrophage was 2.1 and 2, respectively. Figures 6 to 9 display more comprehensive data.

Promastigote Assay
It has been found that longer exposure times and higher MgO NP concentrations have more toxic effects on *L. tropica* and *L. infantum* promastigotes. As a result, the fatal effects of MgO NPs were dose- and time-dependent (Figures 10 and 11). The effects of different MgO NP concentrations on the number of *L. tropica* and *L. infantum* promastigotes were evaluated after incubation for 24, 48, and 72 hours (Figures 10 and 11). In this test, differences were found between all MgO NP concentrations and control groups (*P* < 0.05). The results showed that increasing the concentration of MgO NPs could significantly reduce the proliferation of *L. tropica*.

**Figure 4.** Flow Cytometry Analysis for *Leishmania infantum*. Note. MgO: Magnesium oxide; NP: Nanoparticle. This test was conducted to determine the viability percentage (live percentage) and apoptotic percentage of macrophages in exposure to a 40 μg/mL concentration of MgO NPs. Panel A shows that 30.5% of *Leishmania infantum* parasites underwent apoptosis at a 40 μg/mL concentration of MgO NPs, and 69.4% of *Leishmania infantum* parasites are alive in exposure to this concentration of MgO NPs. Further, Panel B displays that in the untreated control group, 98.0% of *Leishmania infantum* parasites are alive. Further, the upper and lower right sections show secondary and primary apoptosis, respectively. Moreover, the upper and lower left sections depict necrosis and alive cells, respectively.

**Figure 5.** Flow Cytometry Analysis for *Leishmania tropica*. Note. MgO: Magnesium oxide; NP: Nanoparticle. This test was performed to determine the viability percentage (live percentage) and apoptotic percentage of macrophages in exposure to a 40 μg/mL concentration of MgO NPs. Panel A depicts a 40 μg/mL concentration of MgO NPs. Based on the results, 38.56% of *Leishmania tropica* parasites underwent apoptosis, and 56.9% of *Leishmania tropica* parasites are alive in exposure to this concentration of MgO NPs. Moreover, Panel B illustrates that in the untreated control group, 98.4% of *Leishmania tropica* parasites are alive. Moreover, the upper and lower right sections display secondary and primary apoptosis, respectively. In addition, the upper and lower left sections show necrosis and alive cells, respectively.
Today, some drugs such as miltefosine, paromomycin, meglumine antimonate (Glucantime), sodium stibogluconate (Pentostam), amphotericin B, and pentamidine are used for the treatment of \textit{L. infantum} and \textit{L. tropica} (visceral and cutaneous leishmania, respectively).\textsuperscript{8,11} However, these drugs have many side effects such as toxicity, painful injections, and long-term courses of treatment. Additionally, these drugs do not exist enough, are expensive, and on the other hand, some Leishmania spp. have resistance to these drugs.\textsuperscript{3,4,11,22,43,44} Macrophages are one of the most important immune cells and are the most important reservoir of \textit{Leishmania} spp. \textit{Leishmania} inhibits the killing activity of macrophages and multiples in the phagosomes of macrophages and disrupts these cells.\textsuperscript{14-19} To eliminate all these problems, we need to find drugs that have fewer side effects, are cheaper and more available, and, on the other hand, remove the reservoirs of parasites from macrophages. One of these compounds is MgO NP.\textsuperscript{61}

\begin{figure}[h]
\centering
\includegraphics[width=0.45\textwidth]{figure6}
\caption{Amastigote Assay for \textit{Leishmania infantum}. Note. MgO: Magnesium oxide; NP: Nanoparticle. This test was conducted to evaluate the number of \textit{Leishmania infantum} amastigotes in each macrophage. As shown, at 40 and 20 μg/mL concentrations of MgO NPs, the number of \textit{Leishmania infantum} amastigotes in each macrophage was higher than that treated with Glucantime (the treated control group) while lower than the untreated control group.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.45\textwidth]{figure8}
\caption{Amastigote Assay for \textit{Leishmania tropica}. Note. MgO: Magnesium oxide; NP: Nanoparticle. This test was conducted to evaluate the number of \textit{Leishmania tropica} amastigotes in each macrophage. At 40 and 20 μg/mL concentrations of MgO NPs, the number of \textit{Leishmania tropica} amastigotes in each macrophage was higher than those treated with Glucantime (the treated control group) while lower than the untreated control group.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.45\textwidth]{figure7}
\caption{Amastigote Assay for \textit{Leishmania infantum}. Note. MgO: Magnesium oxide; NP: Nanoparticle. This test was performed to investigate the contaminated macrophage percentage with \textit{Leishmania infantum} amastigote. Based on the result, at 40 and 20 μg/mL concentrations of MgO NPs, 11% and 20% of macrophages, respectively, were contaminated with \textit{Leishmania infantum} amastigotes; this percentage in the untreated control group was 40%. Additionally, at these concentrations of MgO NPs, there are more amastigotes within each macrophage than the glucantime (the treated control group).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.45\textwidth]{figure9}
\caption{Amastigote Assay for \textit{Leishmania tropica}. Note. MgO: Magnesium oxide; NP: Nanoparticle. This test was performed to study the contaminated macrophage percentage with \textit{Leishmania tropica} amastigote. At 40 and 20 μg/mL concentrations of MgO NPs, 15% and 24% of macrophages were contaminated with \textit{Leishmania tropica} amastigotes, respectively, and this percentage in the untreated control group was 40%. In addition, at these concentrations of MgO NPs, there are more amastigotes within each macrophage than the glucantime (the treated control group).}
\end{figure}

and \textit{L. infantum} promastigotes. Concentrations of 200 μg/mL and 400 μg/mL of MgO NPs with incubation times of 24, 48, and 72 hours demonstrated the greatest efficacies, whereas concentrations of 0.39 μg/mL and 0.78 μg/mL with incubation times of 24 and 48 hours represented the least efficacy in inhibiting the proliferation and mobility of \textit{L. tropica} and \textit{L. infantum} promastigotes, respectively.

\textbf{Determination of the Half-maximal Inhibitory Concentration}

After 72 hours, the IC\textsubscript{50} value of MgO NPs was 7.32 μg/mL and 12.58 μg/mL for \textit{L. tropica} and \textit{L. infantum}, respectively, which was determined by promastigote assay. Figures 12 and 13 depict the related data.

\textbf{Discussion}

Leishmaniasis is a serious issue in tropical and subtropical areas worldwide.\textsuperscript{59,60} Today, some drugs such as miltefosine, paromomycin, meglumine antimonate (Glucantime), sodium stibogluconate (Pentostam), amphotericin B, and pentamidine are used for the treatment of \textit{L. infantum} and \textit{L. tropica} (visceral and cutaneous leishmania, respectively).\textsuperscript{4,11} However, these drugs have many side effects such as toxicity, painful injections, and long-term courses of treatment. Additionally, these drugs do not exist enough, are expensive, and on the other hand, some Leishmania spp. have resistance to these drugs.\textsuperscript{3,4,11,22,43,44} Macrophages are one of the most important immune cells and are the most important reservoir of \textit{Leishmania} spp. \textit{Leishmania} inhibits the killing activity of macrophages and multiples in the phagosomes of macrophages and disrupts these cells.\textsuperscript{14-19} To eliminate all these problems, we need to find drugs that have fewer side effects, are cheaper and more available, and, on the other hand, remove the reservoirs of parasites from macrophages. One of these compounds is MgO NP.\textsuperscript{61}
This research aimed to assess the anti-leishmanial activity of MgO NPs on *L. tropica* and *L. infantum*-infected macrophages.

Gutiérrez et al discovered that NPs such as MgO NPs have high anti-leishmanial activity on CL and VL forms of leishmaniasis. Allahverdiyev et al and Gutiérrez et al expressed that the effect of MgO NPs and other NPs is probably through an increase in NO (nitric oxide) and ROS production in macrophages. Likewise, Alti et al discussed that gold-Ag bimetallic NPs (Au-Ag BNPs) stimulate the ROS-mediated apoptosis-like death in the *Leishmania* promastigotes, thus these products have anti-Leishmania effects.

In other studies performed by Jebali et al, it was shown that some Ag NPs such as MgO NPs have anti-leishmanial activity on CL and have an activation performance on macrophages through increasing the production of NO and ROS.

In another study, Jebali et al found that MgO NPs and other MgO NPs coated with lectins have anti-leishmanial activity on CL and VL and have macrophage activation performance to kill parasites through increased \( \text{H}_2\text{O}_2 \) and NO and some ILs. Furthermore, the MTT assay and promastigote assay for *L. tropica* and *L. infantum* in our study revealed that MgO NPs have an anti-leishmanial activity on *L. tropica* and *L. infantum* at high concentrations.

Similarly, in this study, it was demonstrated that MgO NPs have the highest toxic efficacy on *L. tropica* and *L. infantum* and macrophages at the 500 µg/mL
In the study of Jebali et al, the results of the MTT test represented that at a concentration of 500 µg/mL, MgO NPs have cytotoxicity and anti-proliferation effects on L. promastigote and cytotoxicity effects on macrophages.\textsuperscript{61} In addition, in the mentioned study and another study by Goonoo et al, according to the flow cytometry method, non-coated MgO NPs could produce ROS that leads to apoptosis.\textsuperscript{61,64} Our flow cytometry test results showed that at the concentration of 40 µg/mL of MgO NPs, 38.56% of L. tropica parasites underwent apoptosis (a combination of primary and secondary apoptosis). Further, 30.5% of L. infantum parasites underwent apoptosis (a combination of primary and secondary apoptosis).

Finally, in some studies,\textsuperscript{27,64} in a dose-dependent (25, 50, 100, and 200 µg/mL) and time-dependent (24, 48, and 72 hours post incubation) assay, with increasing the concentration, MgO NPs could decrease the number of amastigotes (inside of macrophages) and promastigotes in vitro.\textsuperscript{61,64} In our study, at the 20 and 40 µg/mL concentrations of MgO NPs, the percentage of infected macrophages with L. infantum amastigotes was 24% and 15%, respectively, and 40% of macrophages were contaminated with L. tropica and infantum amastigotes in the untreated control group. Finally, at the 20 µg/mL concentration of MgO NPs, the mean number of L. tropica and L. infantum in the infected macrophage was 2.1 and 2, respectively.
Conclusion

The development of novel therapies for CL and VL now appears to be more feasible than before. MgO NPs had a potentially fatal impact on *L. tropica/infantum* promastigotes and amastigotes, as well as the ability to induce apoptosis in these parasites and decrease a load of parasites from leishmania-infected macrophages. In the future, these NPs might be candidates for treating CL and VL.

Acknowledgments

We are highly grateful to Dr. Mohamad Reza Razavi for his helpful consultation and comments on the manuscript.

Authors’ Contribution

Conceptualization: Amir Karimipour-Saryazdi.

Data curation: Roya Omidi.

Formal analysis: Amir Karimipour-Saryazdi.

Funding acquisition: Amir Karimipour-Saryazdi.

Investigation: Mohammad Mahdi Jafari.

Methodology: Fatemeh Ghaffarifar.

Project administration: Fatemeh Ghaffarifar.

Resources: Fatemeh Ghaffarifar.

Software: Seyed Hojaj Sadeghi.

Supervision: Amir Karimipour-Saryazdi.

Validation: Amir Karimipour-Saryazdi.

Visualization: Amir Karimipour-Saryazdi.

Writing–original draft: Mohammad Mahdi Jafari.

Writing–review & editing: Fatemeh Ghaffarifar.

Competing Interests

The authors declare no conflict of interests.

Ethical Approval

Not applicable.

References


10. Sadeghi SH, Neamani S, Moradi L. Immobilization of CdCl2 on filamentous silica nanoparticles as an efficient catalyst for the solvent free synthesis of some amidoalkyl derivatives.


