Efficacy of green synthesized silver nanoparticles via ginger rhizome extract against *Leishmania major* in vitro

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**Abstract**

**Introduction**

Leishmaniasis is a major public health problem that causes by parasite of the genus *Leishmania*. The pentavalent antimonial compounds that used for treatment are not safe or effective enough. The aim of the present study was preparation and evaluation of the efficacy of green synthesized silver nanoparticles against *Leishmania major* (*L. major*) in vitro.

**Methods**

To synthesis silver (Ag) nanoparticles (NPs), ginger extract was added to the 0.2mM AgNO$_3$ aqueous solution (1:20). Effects of different concentrations of Ag-NPs on the number of *L. major* promastigotes were investigated using counting assay. The MTT test was applied to determine the toxicity of Ag-NPs on promastigotes of *L. major*, as well as, macrophage cells. Then, to evaluate the anti-amastigote effects of Ag-NPs, parasites within the macrophages were counted by light microscope. Furthermore, to determine the induced apoptosis and necrotic effects of Ag-NPs on promastigotes, flow cytometry method was employed using annexin staining.

**Results**

The effect of Ag-NPs on promastigotes and amastigotes of *L. major* was effective and has a reverse relationship with its concentration. According to the results of anti-amastigote assay, the IC50 value of this nanoparticle was estimated 2.35 ppm after 72h. Also, Ag-NPs caused Programmed Cell Death (PCD) in promastigotes of *L. major* and showed 60.18% of apoptosis.

**Discussion**

Based on the mentioned results, it can be concluded that Ag NPs has a beneficial effect on promastigote and amastigote forms of *L. major* in vitro. Hence, these nanoparticles could be applied as promising antileishmanial agents for treatment of Leishmania infections.
Introduction

Leishmaniasis is still one of the major global health problems that causes by parasite of the genus Leishmania and can be transmitted through the bite of female phlebotomine sandflies in tropical and subtropical climates regions [1, 2]. The poorest are at the highest risk of morbidity and mortality in five continents of the World. Due to Leishmania parasites Clinical manifestations occur in at least three different forms, such as cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). Based on the diverse clinical forms of cutaneous leishmaniasis, the consequent complications will be different ranging from self-limited skin disease to diffuse (severe) visceral disease [3, 4]. On average, approximately 350 million people are estimated at risk of leishmaniasis and 12 million people have become infected throughout the world. Noteworthy, this skin infection is prevalent in 98 countries. Every year, there are more than 1 million new cases of cutaneous leishmaniasis and 500,000 new cases of visceral leishmaniasis among people in developed and developing countries, according WHO reports [5, 6]. Despite efforts of scientists, there is no effective prevention and treatment strategy against leishmaniasis. Over the last 70 years, chemotherapy has still remained the most popular treatment for leishmaniasis diseases, as well as, antimony based drugs, such as sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime) have affected the highest percentage of cures [7–9]. Also, it has been demonstrated that these medications are able to prevent the production of adenosine triphosphate (ATP) via interruption in phosphokinase enzyme activity [9]. Accumulating evidence has shown that drugs used for leishmaniasis have not been completely successful yet, and also have several disadvantages such as low sensitivity, non-specific, toxicity, financial burden, long-term treatment, drug and parasite resistance, painful administration route, treatment failures, as well as damaging some tissues [9–11]. Since antiparasitic drugs have unwilling side effects and also may cause serious problems, discovering and development of appropriate treatment methods or potent antileishmania compounds are needed [12]. Currently, the medical application of nanoparticles (NPs) [13–16] is an exciting innovation that complements the global health community’s efforts to end the leishmaniasis endemics. For instance, chitosan, Au, Ag, Fe$_3$O$_4$, TiO$_2$ and ZnO were employed to treat the diseases are related $L$. major [17, 18]. Moreover, some of the plants extracts like Aloe-emodin, Artemisinin, Thymus migricus and Tussilago farfara were used to remedy leishmania parasite [19–21]. The previous researches have been shown that Silver nanoparticles have antileishmanial effects [22–25].

In this research, based on proved antimicrobial behavior of Ag NPs [26–29], as well as according to antileishmania activity of silver [30], as well as antimicrobial nature of ginger extract [31], the silver NPs synthesized via ginger extract to evaluate the therapeutic effects against $L$. major for the first time according to the best of our knowledge. In this respect, silver NPs prepared in a green route through ginger extract initially, followed by characterizing via UV-visible spectroscopy and TEM images.

Materials and methods

Extract preparation and synthesis of silver nanoparticles

In this study, extracts and silver nanoparticles prepared according to reported investigation on green synthesis of silver nanoparticles [19]. In this regard, in the first step, ginger rhizome was washed by deionized (DI) water, followed by slicing to the fine pieces, after which exposed to a shadow at 27°C for four days to become completely dry. Afterward, 0.2 g of fine gingers was poured into 100 mL DI water, stirring at 80°C for 40 minutes. Furthermore, the extract was filtered by Whatman No.1 paper and centrifuged for 5 minutes at 4000 rpm, before passing...
through a 0.22 syringe filter, and maintained at 4°C far from the light. To synthesis silver NPs, the 0.2mM AgNO$_3$ solution prepared initially, after which the extract was putted on the AgNO$_3$ aqueous solution (1:20). First, the solution was approximately colorless and by progression of the reaction, the color changed from the light yellow to dark brown, which could be a visual attest to confirm formation of silver NPs. Next, by employing a 12 kD dialysis bag, the silver NPs dialyzed in water for 24 h, and filtered via 0.22μm syringe filters.

**Characterization of Ag NPs**

In the first step of characterization, a SPUV-26 SC-Tech spectrophotometer was employed to carry out the UV–visible spectroscopy to assess formation of green synthesized silver NPs. Moreover, to evaluate the size and shape of nanoparticles, Transmission electron microscopy (TEM) method was carried out via Leo 912 AB microscope.

**Collection and cultivation of parasites**

In this work, *L. major* Iranian standard strain promastigotes (MRHO/IR/75/ER) were obtained from parasitology department of Tarbiat Modares University. For growth and replication of the *L. major* promastigotes the nutrient RPMI 1640 medium enriched with penicillin (100 unit ml$^{-1}$), streptomycin (100 μgml$^{-1}$) fetal calf serum (10% v/v) were used and after that incubated in 25 ± 1°C.

**Culturing of macrophage cells**

The study used macrophages were derived from RAW.264.7 macrophage cell line which is obtained by the Parasitology Department at Tarbiat Modares University of Tehran, Iran. At the beginning, RAW.264.7 macrophages were cultured in RPMI 1640 containing 10% FBS (Gibco-BRL, France), penicillin (100 unit ml$^{-1}$), and streptomycin (100 μgml$^{-1}$) (Sigma Chemical Co.), then these culture flasks incubated in a humidified atmosphere of 95% air and 5% CO$_2$ at 37°C.

**Promastigote assay**

For this assay, 100 μL of promastigotes (2 × 10$^6$ cell/mL) was seeded in 96-well plate containing 100 μL of RPMI1640 pluse 15% FBS in the presence of several concentrations (40, 20, 10, 5, 2.5, 1.25, 0.625 and 0.312 ppm) of the Ag NPs solution as triplicate and were kept at a temperature of 24 ± 2°C for 24, 48, and 72 hours. In addition, AmpB (GILEAD UK) (1 μg/mL) and Glucantime (Sanofi-Aventis France) (100 μg/mL) were used as positive control groups [32]. At the end, the direct counting method by hemocytometer chamber (Neubauer chamber) was performed to evaluate the anti-leishmanial effects of Ag NPs on promastigotes of *L. major* and the results compared with control groups as well as analyzed by Graph pad Prism 5.0 [16].

**Cytotoxic effects of green synthesized silver nanoparticles on *L. major* promastigotes**

In order to assessing the antileishmanial effects of silver NPs upon the promastigotes of *L. major* parasites, 3- (4, 5-dimethylthiazol- 2-yl) -2, 5-diphenyl-tetrazolium bromide (MTT, Sigma-Aldrich) assay was taken. Briefly, in 96-well microtitr plates *L. major* promastigotes (100 μl, 1×105 cell ml$^{-1}$) were cultivated in the presence of different amounts of silver NPs (40 to 0.16 ppm) for 24, 48, and 72 h at 26°C. After that, 20 μL of MTT reagent (5 mg/mL) was added to the wells and further incubated for 3–5 hours in the incubator. Subsequently, they centrifuged for 10 minutes at 3000 rpm. For observation of formazan crystals the contents of
each well was removed and replaced with 100 μL of dimethyl sulfoxide (DMSO) and then kept at room temperature for 30 minutes. Eventually, using an ELISA plate reader (ELX800), absorbances were read within 30 minutes at a wavelength of 570 nm and the results analyzed by GraphPad prism5 software to determine IC50 of Leishmania growth. The percentage of viability was measured using the following formula:

\[
\text{Cell Viability} \% = \frac{(\text{Drug well absorption}) - (\text{Blank well absorption})}{(\text{Control well absorption}) - (\text{Blank well absorption})} \times 100
\]

Cytotoxic effects of green synthesized silver nanoparticles on macrophages

In the present study, for estimation of uninfected macrophage viability, RAW264.7 macrophages which were grown and propagated in complete RPMI1640 containing 10% FBS were harvested and cultured in 96-well culture plates (100 μL, 1×10^5 cell mL^{-1}) with different concentrations of silver NPs (40 to 0.16 ppm). The samples were placed in the incubator for three days at 37°C in a 5% CO₂. After incubation period, MTT assay was performed as mentioned above.

Amastigote assay

At the beginning, Murine macrophage-derived RAW 264.7 cells (1×10^5 cell ml−1) were seeded into the 12-well micro-plates with rounded coverslips on the bottom, then the samples were subsequently incubated overnight at 37°C in a CO₂ incubator (5% CO₂ and 95% relative humidity). In this context, non-adherent macrophages were omitted thoroughly with the use of cold phosphate buffer saline (PBS) whereas adherent macrophages were infected through the stationary phase of L. major Promastigotes (at 10:1 ratio of parasites/macrophages). Following incubation for 24 h at 37°C with 5% CO₂, the plate was again washed (as above) and excess cells were eliminated. Then, infected macrophages were treated with different volumes of silver NPs (1.25 and 2.5 ppm) and were incubated in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. Moreover, the infected cells in the absence of the drug were employed as a reference of negative control groups. After 24 h incubation, the supernatant was discarded and the coverslips were fixed in methanol to stain with Giemsa solution. Tachyzoites within the macrophages was counted and the results were compared with the untreated control group by using an optical microscope [16].

Flow cytometry analysis

To identify the drug-induced apoptosis and necrosis Annexin-V FLUOS staining kit (Biovision, USA) with annexin V-FITC and PI (propidium iodide) staining was used. In brief, 2×10^6 ml−1 promastigotes were exposed to 5 μg/ mL concentration of Ag NPs and placed in the incubator, as mentioned above. In addition, L. major promastigotes without drug were considered as the control group. 24 hours later, the samples harvested and centrifuged at 3000 rpm for 10 min. After emptying the supernatant liquid, 500μL binding buffer, 5μL annexin V and 5μL propidium iodide (PI) were added to the pellets and incubated at 25°C under dark conditions for 5 min. Absorption of annexin-V by cells was measured by FACSCaliber (FACS-Canto II) and the percentage of apoptotic, necrotic and normal cells for each sample were analyzed using FlowJo Software [33].

Statistical analysis

SPSS software version 21 (SPSS Inc., Chicago, IL, USA) and Graph Pad Prism version 5.0 (GraphPad Software, Inc., San Diego, CA) were applied in order to statistical analysis. All the obtained results were expressed as mean ± S.D and compared by one-way ANOVA as
parametric tests. Additionally, a probability (P) value less than 0.05 (p < 0.05) was regarded as the statistical differences.

**Results**

**UV-visible spectroscopy of silver NPs**

To evaluate the formation of silver NPs, UV-visible spectroscopy of green synthesized solution was carried out against water and the spectrum is shown in Fig 1. The diagram shows a peak between 400 and 460 nm (430 nm), which is the surface plasmon resonance (SPR) of green synthesized silver NPs and confirms formation of silver NPs. The figure illustrate that by increasing the time of reaction, SPR intensity elevates, which it means the formation of higher amounts of silver NPs.

**TEM image of silver NPs**

The shape and size of green synthesized silver NPs is shown in Fig 2A via TEM image. Moreover, the size distribution of NPs is presented in Fig 2B. As it can be seen, the TEM image shows that the NPs are approximately spherical and the mean size of silver NPs is 10 ± 4 nm.
The effect of silver NPs on promastigotes growth

The effects of different concentrations of Ag NPs on the number of *L. major* promastigotes were investigated after incubation for 24, 48, and 72 h (Fig 3). In this test, considerable differences were observed between all concentrations of Ag NPs and control groups (p < 0.05). The results showed that by increasing the concentration of Ag NPs, proliferation of *L. major* promastigotes decreased remarkably. The concentrations of 40, 20, 10, and 5 ppm of Ag NPs with incubation times of 24, 48, and 72 h showed the most efficacies, whereas the concentration of 0.312 and 0.39 ppm after 24 and 48 h incubation, respectively, presented the least efficacies on inhibiting the proliferation and mobility of *L. major* promastigotes. Furthermore, it was found that high concentrations (40, 20, 10 and 5 ppm) of Ag NPs completely inhibit the proliferation of *L. major* promastigotes after 24, 48, and 72 h.

The cytotoxicity of silver NPs to the promastigotes by MTT

Cytotoxicity effect of the silver NPs against *L. major* promastigotes was investigated by optical density (OD) following MTT assay. As Fig 4 shows, parasite viability was found to be based on a dose-dependent response and decreased by increasing the silver NPs concentration. In other words, the maximum toxicity was related to concentration of 40 ppm after 24 h, whereas at concentration of 3.12 ppm of the silver NPs the results were close to the reference drug (AmpB).

The cytotoxicity of silver NPs to macrophages by MTT

It was observed that high concentrations of the silver NPs have more toxic impacts upon macrophage cells than low concentrations compared to the control group (Fig 5).

Anti-amastigote assay

In the current investigation, the anti-amastigote effect of the silver NPs on infected macrophage cells was determined. In this regard, the mean number of amastigotes/macrophage was examined by optical microscope after 72 h of incubation. The percentage of infectivity in the infected macrophages were 14.75 and 27.5 after being treated with 1.25 and 2.5 ppm of silver NPs, respectively, while in the negative control group, 30% of macrophages were infected with amastigotes (Table 1). Additionally, the IC50 value of the silver NPs on macrophage cells was equal to 2.35 ppm for a time period of 72 h.
Fig 4. The viability of *L. major* promastigotes in the presence of different concentrations of the Ag NPs (40 to 0.16 ppm), after 72 h incubation and compared them with the control groups.

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Fig 5. Percentage of viability of the uninfected RAW macrophages with different concentrations of the Ag NPs (40 to 0.16 ppm), after 72 h incubation in comparison to control group.

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Flow cytometry assay
In order to calculate the percentage of necrotic, apoptotic, and alive cells in *L. major* promastigotes flow cytometry assay was employed after staining with Annexin-V and PI.

Here, this study showed that the percentage of apoptosis and necrosis induced in promastigotes exposed to 5 ppm concentrations of silver NPs after 72 h incubation were estimated 60.18% and 0.53%, respectively. However, the percentage of alive cells in the control group (without treatment) was determined 99.59%. The outputs of flow cytometry analysis are represented in Fig 6.

Discussion
Leishmaniasis is now widely remained as one of the main health problems all around the world. Current therapy against leishmaniasis wasn’t satisfactory, and applied drugs (glucantime and pentostam) are not the complete options, because of many side effects, expensive cost, unacceptable toxicity, painful administration, as well as, the appearance of drug resistance in some endemic areas were limited. Accordingly, researchers are continuously seeking to develop and design effective anti-leishmanial agents that could fulfil entirely the treatment goals. In previous studies, the results showed that silver nanoparticles have anti-leishmanial effect [22–25]. Mohebali et al. found with 100 ppm of Nanosilver could damaged 85% of...
infected macrophages with amastigotes of *L. major* [25]. In the present study, the effectiveness of various concentrations of green synthesized silver nanoparticles via ginger rhizome extract against *L. major* promastigotes, as well as amastigotes was investigated. Considerable toxicity has been observed for amastigotes and promastigotes of *L. major* in the presence of the NPs. The findings showed that proliferation of *L. major* promastigotes significantly decreased by increasing the Ag-NPs concentration and exposure time compared to standard drugs, Amphotericin B and Glucantim. In other words, high concentrations (40, 20, 10 and 5 ppm) of Ag NPs completely inhibited the proliferation of *L. major* promastigotes after 24, 48, and 72 h. The current MTT assay results displayed that the percentage viability of macrophage cells and *L. major* promastigotes exposed to NPs decreased in a concentration-dependent manner, compared to the control group. The viability percentages for macrophages and *L. major* promastigotes treated with the maximum concentration of NPs (40 ppm) was reported 7.3% and 32.2%, respectively. The mean number of amastigotes in each macrophage was decreased by 1.25 and 2.5 ppm of Ag-NPs after 72 h of incubation compared with control groups. In addition, the IC50 value against this parasitic form of *L. major* was 2.35 ppm after 72 h exposure. Therefore, it found that these nanoparticles induced the inhibition of the proliferation rate of intramacrophage amastigotes and also can be effective in reducing infected macrophages. As reported in previous studies, silver nanoparticles are able to induce programmed cell death in different cells [34–36]. Also, our findings showed that regarding to control group (promastigotes without treatment), induction of apoptosis was increased in promastigotes of *L. major* after exposure to 5 ppm nanoparticles, and hence these apoptotic effects were appreciable. Therefore, it is inferred that Ag-NPs could be effective against *L. major* by inducing apoptosis. Many studies reported the positive effects of silver nanocomposites into different microorganisms and some diseases [37]. In this regard, cytotoxic effect of these nanocomposites against bacteria, viruses, fungi and different types of cancer has been examined, and results demonstrated that silver nanocomposites has satisfactory levels of cytotoxicity effect against these cancers and microorganisms [17, 37, 38]. Although a number of studies have demonstrated the antimicrobial properties of silver nanocomposites in some parts of the world, more investigations are needed to better understand of anti-parasitic activities of these nanomaterials against leishmania parasite. In a study conducted by Mohebali and colleagues, the antileishmania activities of silver nanoparticles on *L. major* were investigated in vitro and in vivo conditions. According to their results, silver nanoparticles reduced proliferation of amastigote stages of *L. major* same as the reference drug. Also, they concluded that nano-silver can be useful to prevent secondary infection in cutaneous leishmaniasis caused by *L. major* [25].

In another study, the effect of silver (Ag) NPs at the presence of ultraviolet (UV) light led to a reduction in the metabolic activity and proliferation rates of *Leishmania tropica* [23].

In the Jebali and Kazemi (2013) study, they assessed antileishmanial effects of five nanoparticles including (ZnO NPs, Au NPs, TiO2 NPs and etc.) on *L. major* under invisible ultraviolet (UV), infrared light(IR) and dark conditions. Results revealed that all of the nanoparticles had inhibitory effects on *L. major* parasite, but the greatest antileishmanial activity was related to silver (Ag) NPs during illumination [17]. Based on the above mentioned points, it can be concluded that silver (Ag) NPs can be applied as promising antileishmanial agents for treatment of Leishmania infections.

**Conclusion**

Recently, the use of nanoparticles has continuously increased because of their unique structural characteristics for the treatment of various diseases. The results in this study indicated acceptable level of in vitro activity of silver (Ag) NPs against *L. major* promastigotes as well as
intracellular amastigotes. Also, the results of flow cytometry demonstrated that (Ag) NPs can cause Programmed Cell Death (PCD) in promastigotes of *L. major* and showed 60.18% of apoptosis in the exposed group of promastigotes. However, the research suggests that further efforts should be done in order to identification of antileishmanial activities of these nanoparticles under in vivo condition.

**Supporting information**

S1 Data. (DOCX)

S2 Data. (DOCX)

S3 Data. (DOCX)

S4 Data. (DOCX)

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**References**

1. Desjeux P. Leishmaniasis: current situation and new perspectives. Comparative immunology, microbiology and infectious diseases. 2004 Sep 1; 27(5):305–18. https://doi.org/10.1016/j.cimid.2004.03.004 PMID: 15225961

2. Karimkhani C, Wang V, Coffeng LE, Naghavi P, Dellavalle RP, Naghavi M. Global burden of cutaneous leishmaniasis: a cross-sectional analysis from the Global Burden of Disease Study 2013. The Lancet Infectious Diseases. 2016 May 1; 16(5):584–91. https://doi.org/10.1016/S1473-3099(16)00003-7 PMID: 26879176

3. Abdoli A, Maspi N, Ghaffarifar F. Wound healing in cutaneous leishmaniasis: a double edged sword of IL-10 and TGF-β. Comparative immunology, microbiology and infectious diseases. 2017 Apr 1; 51:15–26. https://doi.org/10.1016/j.cimid.2017.02.001 PMID: 28504090

4. Akhoundi M, Kuhlis K, Cannet A, Volýpka J, Marty P, Delaunay P, et al. A historical overview of the classification, evolution, and dispersion of Leishmania parasites and sandflies. PLoS neglected tropical diseases. 2016 Mar 3; 10(3):e0004349. https://doi.org/10.1371/journal.pntd.0004349 PMID: 26937644

5. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. WHO Leishmaniasis Control Team. Leishmaniasis worldwide and global estimates of its incidence. PloS one. 2012 May 31; 7(5):e35671. https://doi.org/10.1371/journal.pone.0035671 PMID: 22693548

6. Sosa N, Pascale JM, Jiménez AI, Norwood JA, Kreishman-Detrick M, Weina PJ, et al. Topical paromomycin for New World cutaneous leishmaniasis. PLoS neglected tropical diseases. 2019 May 2; 13(5):e0007253. https://doi.org/10.1371/journal.pntd.0007253 PMID: 31048871
7. de Menezes JP, Guedes CE, Petersen AL, Fraga DB, Veras PS. Advances in development of new treatment for leishmaniasis. BioMed research international. 2015 Oct;2015. https://doi.org/10.1155/2015/815023 PMID: 26078965

8. Singh N, Kumar M, Singh RK. Leishmaniasis: current status of available drugs and new potential drug targets. Asian Pacific journal of tropical medicine. 2012 Jun 1; 5(6):485–97. https://doi.org/10.1016/S1995-7645(12)60084-4 PMID: 22575984

9. Tiuman TS, Santos AO, Ueda-Nakamura T, Dias Filho BP, Nakamura CV. Recent advances in leishmaniasis treatment. International Journal of Infectious Diseases. 2011 Aug 1; 15(8):e525–32. https://doi.org/10.1016/j.ijid.2011.03.021 PMID: 21605997

10. Maspi N, Ghaffarifar F, Sharifi Z, Dalimi A, Dayer MS. Immunogenicity and efficacy of a bivalent DNA vaccine containing LeIF and TSA genes against murine cutaneous leishmaniasis. Aprmis. 2017 Mar; 125(3):249–58. https://doi.org/10.1111/apm.12651 PMID: 28323431

11. Sundar S, More DK, Singh MK, Singh VP, Sharma S, Makharia A, et al. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. Clinical infectious diseases. 2000 Oct 1; 31(4):1104–7. https://doi.org/10.1086/318121 PMID: 11049798

12. Croft SL, Seifert K, Yardley V. Current scenario of drug development for leishmaniasis. The Indian journal of medical research. 2006; 123(3):399–410. PMID: 16778319

13. Mohammad M, Tavajohi A, Ziashahabi A, Pournoori N, Muhammadnejad S, Delavari H, et al. Toxicity, morphological and structural properties of chitosan-coated Bi2O3–Bi(OH)3 nanoparticles prepared via DC arc discharge in liquid: a potential nanoparticle-based CT contrast agent. Micro & Nano Letters. 2019 Mar 6; 14(3):239–44.

14. Tripathi KM, Ahn HT, Chung M, Le XA, Saini D, Bhati A, et al. N, S, and P-Co-doped Carbon Quantum Dots: Intrinsic Peroxidase Activity in a Wide pH Range and Its Antibacterial Applications. ACS Biomaterials Science & Engineering. 2020 Sep 3; 6(10):5527–37.

15. Bajpai VK, Khan I, Shukla S, Kang SM, Aziz F, Tripathi KM, et al. Multifunctional NP-doped carbon dots for regulation of apoptosis and autophagy in B16F10 melanoma cancer cells and in vitro imaging applications. Theranostics. 2020; 10(17):7841. https://doi.org/10.7150/thno.42291 PMID: 32685024

16. Bajpai VK, Khan I, Shukla S, Kumar P, Chen L, Anand SR, et al. P-Doped Carbon Nanodots for Food-Matrix Decontamination, Anticancer Potential, and Cellular Bio-Imaging Applications. Journal of Biomedical Nanotechnology. 2020 Mar 1; 16(3):283–303. https://doi.org/10.1166/jbn.2020.2899 PMID: 32493540

17. Jebali A, Kazemi B. Nano-based antileishmanial agents: a toxicological study on nanoparticles for future treatment of cutaneous leishmaniasis. Toxicology in vitro. 2013 Sep; 27(6):1896–904. https://doi.org/10.1016/j.tiv.2013.06.002 PMID: 23806227

18. Tavakoli P, Ghaffarifar F, Delavari H, Shahpari N. Efficacy of manganese oxide (Mn2O3) nanoparticles against Leishmania major in vitro and in vivo. Journal of Trace Elements in Medicine and Biology. 2019 Dec 1; 56:162–8. https://doi.org/10.1016/j.jtemb.2019.08.003 PMID: 31473959

19. Mohammad M, Shahisaraee SA, Tavajohi A, Pournoori N, Muhammadnejad S, Mohammadi SR, et al. Green synthesis of silver nanoparticles using Zingiber officinale and Thymus vulgaris extracts: characterisation, cell cytotoxicity, and its antifungal activity against Candida albicans in comparison to fluconazole. IET nanobiotechnology. 2018 Sep 7; 12(2):114–9.

20. Ghafarifar F, Heydari FE, Dalimi A, Hassan ZM, Delavari M, Mikaeiloo H. Evaluation of apoptotic and antileishmanial activities of Artemisinin on promastigotes and BALB/C mice infected with Leishmania major. Iranian journal of parasitology. 2015 Apr; 10(2):258. PMID: 26246824

21. Soosaraei M, Fakhar M, Teshnizi SH, Hezarjaribi HZ, Baninostafavi ES. Medicinal plants with promising antileishmanial activity in Iran: a systematic review and meta-analysis. Annals of medicine and surgery. 2017 Sep 1; 21:63–80. https://doi.org/10.1016/j.amsu.2017.07.057 PMID: 28794869

22. Dalimi A, Karimi M, Jamele F, Ghafarifar F, Dalimi A. The killing in vitro effect of Half-Wave Rectified Sine electricity plus silver nanoparticle on Leishmania major promastigotes and BALB/C mice skin leishmanial lesion. Tropical Biomedicine 2018; 35 (1), 50–58. PMID: 33601776

23. Allahverdiyev A.M., Sefik, Abamor E., Bagirova M., Ustundag C.B., Kaya C., Kaya F. et al. (2011). Antileishmanial effect of silver nanoparticles and their enhanced antiparasitic activity under ultraviolet light. International Journal of Nanomedicine 6: 2705–2714. https://doi.org/10.2147/IJN.S239885 PMID: 22114501

24. Baiocco P., Ilari A., Ceci P., Orsini S., Grammiccia M., Di Muccio T. et al. (2011). Inhibitory effect of silver nanoparticles on trypanothione reductase activity and Leishmania infantum proliferation. ACS Medicinal Chemistry Letters 2: 230–233. https://doi.org/10.1021/ml1002629 PMID: 24900299

25. Mohesbali M., Rezayat M.M., Gilani K., Sarkar S., Akhoundi B., Esmaeili J., et al. (2009). Nanosilver in the treatment of localized cutaneous leishmaniasis caused by Leishmania major (MRHO/IR/75/ER): an in vitro and in vivo study. Daru 17(4): 285–289.
26. Mohan A, Dipallini S, Lata S, Mohanty S, Pradhan PK, Patel P, et al. Oxidative stress induced antimicrobial efficacy of chitosan and silver nanoparticles coated Gutta-percha for endodontic applications. Materials Today Chemistry. 2020 Sep 1; 17:100299.

27. Verma SK, Jha E, Panda PK, Thirumurugan A, Patro S, Parashar SK, et al. Molecular insights to alkaline based bio-fabrication of silver nanoparticles for inverse cytotoxicity and enhanced antibacterial activity. Materials Science and Engineering: C. 2018 Nov 1; 92:807–18. https://doi.org/10.1016/j.msec.2018.07.037 PMID: 30184810

28. Husain S, Verma SK, Azam M, Sardar M, Haq QM, Fatma T. Antibacterial efficacy of facile cyanobacterial silver nanoparticles inferred by antioxidant mechanism. Materials Science and Engineering: C. 2021 Mar 1; 122:111888. https://doi.org/10.1016/j.msec.2021.111888 PMID: 33641896

29. Verma SK, Jha E, Panda PK, Mishra A, Thirumurugan A, Das B, et al. Rapid novel facile biosynthesized silver nanoparticles from bacterial release induce biogenicity and concentration dependent in vivo cytotoxicity with embryonic zebrafish—A mechanistic insight. Toxicological Sciences. 2018 Jan 1; 161 (1):125–38. https://doi.org/10.1093/toxsci/kfx204 PMID: 29029321

30. Dolat E, Rajabi O, Salariabadi SS, Yadegari-Dehkordi S, Sazgarnia A. Silver nanoparticles and electroporation: their combinational effect on Leishmania major. Bioelectromagnetics. 2015 Dec; 36(8):586–96. https://doi.org/10.1002/bem.21945 PMID: 26769083

31. Liu M, Teng CP, Win KY, Chen Y, Zhang X, Yang DP, et al. Polymeric encapsulation of turmeric extract for bioimaging and antimicrobial applications. Macromolecular rapid communications. 2019 Mar; 40 (5):1800216. https://doi.org/10.1002/marc.201800216 PMID: 30085362

32. Abazari R, Mahjoub AR, Molaie S, Ghaffarifar F, Ghasemi E, Slawin AM, Carpenter-Warren CL. The effect of different parameters under ultrasound irradiation for synthesis of new nanostructured Fe3O4@bio-MOF as an efficient anti-leishmanial in vitro and in vivo conditions. Ultrasonics sonochemistry. 2018 May 1; 43:248–61. https://doi.org/10.1016/j.ultsonch.2018.01.022 PMID: 29555282

33. KarimiPour Saryazdi A, Ghaffarifar F, Dalimi A, Dayer MS. In-vitro and in-vivo comparative effects of the spring and autumn-harvested Artemisia aucheri Bioss extracts on Leishmania major. Journal of ethnomedicine. 2020; 257:112910. https://doi.org/10.1016/j.jep.2020.112910 PMID: 32344159

34. Almalki MA, Khalifa AY. Silver nanoparticles synthesis from Bacillus sp KFU36 and its anticancer effect in breast cancer MCF-7 cells via induction of apoptotic mechanism. Journal of Photochemistry and Photobiology B: Biology. 2020 Mar 1; 204:111786. https://doi.org/10.1016/j.jphotobiol.2020.111786 PMID: 31982671

35. Liao S, Zhang Y, Pan X, Zhu F, Jiang C, Liu Q, et al. Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant Pseudomonas aeruginosa. International journal of nanomedicine. 2019; 14:1469. https://doi.org/10.2147/IJN.S191340 PMID: 30880959

36. Zahir AA, Chauhan IS, Bagavan A, Kamaraj C, Elango G, Shankar J, et al. Green synthesis of silver and titanium dioxide nanoparticles using Euphorbia prostrata extract shows shift from apoptosis to G0/G1 arrest followed by necrotic cell death in Leishmania donovani. Antimicrobials agents and chemotherapy. 2015 Aug 1; 59(8):4782–99. https://doi.org/10.1128/AAC.00098-15 PMID: 26033724

37. Siddiqi KS, Husen A, Rao RA. A review on biosynthesis of silver nanoparticles and their biocidal properties. Journal of nanobiotechnology. 2018 Dec; 16(1):1–28. https://doi.org/10.1186/s12951-017-0328-8 PMID: 29321058

38. El-Khadragy M, Alolayan EM, Metwally DM, El-Din MF, Alobud SS, Alsultan NJ, et al. Clinical efficacy associated with enhanced antioxidant enzyme activities of silver nanoparticles biosynthesized using Moringa oleifera leaf extract, against cutaneous leishmaniasis in a murine model of Leishmania major. International journal of environmental research and public health. 2018 May; 15(5):1037.