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In Vitro Efficacy of Silver Metallic Nanoparticles on Cytotoxic Phagocytic Activity of Macrophages to Promastigote of Leishmania Tropica

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Abstract

Neglected tropical diseases (NTDs) are on the rise due to number of factors, including unplanned urbanisation, international travel and trade, and climate change. The female Lutzomyia sandfly bite is the means of contracting cutaneous leishmaniasis, one of the NTDs. The toxicity of macrophages in response to silver nanoparticles against *L. tropica promastigotes* was estimated by using four replicates were employed for each concentration to guarantee precise results, and serially attenuated concentrations (0.5, 1, 2 µg/mL) were employed. The viability of macrophages and *Leishmania* in the promastigote stage was verified using the MTT assay colorimetric test that generates a distinct colour change when living cells are present. The viability of the cells is measured at 586 nm using a wavelength reader. The results suggested that silver nanoparticles were effective in reducing the proliferation of promastigotes. The most significant antiparasitic effects were demonstrated by Ag NPs, which substantially reduced parasite viability at the lowest concentration (0.5 µg/mL), absorbance reduction from control 0.249 to 0.076) nm.

Keywords: Silver nanoparticles, *leishmania tropica*, MTT assay.

1. Introduction

Climate change, unplanned urbanisation, and international travel and commerce are all contributing to the rise of neglected tropical diseases (NTDs). Among the NTDs, *cutaneous leishmaniasis* (CTD) is acquired by the bite of a female *lutzomyia sandfly*. The infection usually starts as an ulcer on the body's most exposed parts, such as the face, neck, arms, and legs [1].

It may progress to deformities, overlap joints, and cause bacterial or fungal superinfection [1]. Majority of fatalities are linked to side effects from the medications used to treat CL, even though the condition is not fatal in and of itself [2]. The 53 countries where leishmaniasis is endemic, 12 account for 91 % of the reported

worldwide incidence of the disease, with Afghanistan, Brazil, Algeria, and Colombia being the most prominent [3].

Brazil, the sixth most populated and fifth biggest nation in the world by area, is home to a wide range of natural systems and species, but it is also the country that accounts for 72.6 % of all cases of *cutaneous leishmaniasis* (CL) in the Americas [4]. There are currently no vaccinations to prevent human infection, and the most common medications used to treat CL are very toxic: amphotericin B (deoxycholate or liposome) and pentavalent antimonial (*N*-methylglucamine and sodium stibogluconate) [5].

Aside from the fact that some species have become resistant to pentavalent antimonial (Sb5+), no specific medication was completely eradicated the parasite. There are serious side effects linked to its use [6]. Therefore, it is essential to discover less hazardous and more effective therapies for CL. Silver nanoparticles (Ag NPs) are the most potent antimicrobials among noble metal nanoparticles, which are renowned for their low toxicity and excellent selectivity in targeting microbes.

To create a stable and toxic-free silver nanoparticles, organic chemicals are often used as stabilisers. According to certain research, these nanoparticles have strong antileishmanial properties [7]. This study presents the synthesis and characterisation of novel silver nanoparticles, as well as an evaluation of their antileishmanial activity against *leishmania tropica* promastigotes cytotoxicity to macrophages.

2. Materials and Methods

The current study was done at the postgraduate lab, College of Science, Department of Biology, between September 2024 and February 2025.

2.1 Cultures Media

The semi-solid medium was chosen for its ability to maintain the viability and motility of *leishmania* parasites for long periods. A 250 mL formulation containing agar, meat extract, glucose, peptone, inorganic salts, 50 mL defibrinated rabbit blood, and 200 mg gentamicin [8].

Every concentration aside from blood and the antibiotic gentamicin was dissolved in distilled water with a pH of 7.4 ± 0.1. The mixture was cooled to 50 °C in sterile water with mild stirring after being steam sterilised for 15 minutes at 121 °C and 1 bar. The medium was separated into 15 mL sections and placed in sterile 30 mL containers.

Then, incubated for 24 hours at 37 °C to evaluate contamination and sterility, and kept in the refrigerator [9]. The RPMI medium (Capricorn, USA) supplemented with 10 % FBS and 1 % Gentamicin for rapid parasite multiplication.

2.2 Cutaneous Sample

Leishmania tropica viable cultures were prepared and maintained in the lab by continuous subcultures conducted under sterile conditions to avoid microbial contamination. Preparations were made for all materials and media, and the laminar flow hood was sterilised. Sterile culture flasks were placed close to a Bunsen burner flame and filled with 100 mL of RPMI 1640 medium, supplemented with 10 % foetal calf serum and 1 % gentamicin to lower the possibility of airborne contamination.

Each culture flask containing the prepared media was carefully filled with 1 mL of the parasite suspension made from isolated *leishmania tropica* parasites that had been maintained in sterile containers or centrifuge tubes. After being promptly sealed, the culture flasks were incubated at 25 °C. Moreover, to verify the viability of the cultures and identify morphological changes, parasite growth was observed every day under a light microscope, and subcultures were carried out on a regular basis [10].

2.3 Monocyte Culture

An equivalent volume of PBS was utilised to dilute the venous blood specimen. The diluted blood sample was transferred to a centrifuge tube with Ficoll-Paque and spun from 30 to 40 minutes at room temperature at 400 g. Monocytes created a distinct white layer between the plasma and Ficoll-Paque during centrifugation. The monocyte layer was extracted and transferred to a fresh tube. The monocytes were centrifuged at 300 g for 10 minutes to wash with PBS. Following purification, the monocytes were placed in RPMI 1640 culture media supplemented with 10 % FBS and 50 µg/ml gentamicin and incubated at 26 °C [11].

2.3.1 Preparation of Silver nanoparticles (Ag NPs)

Commercial Ag NPs 0.169 g were dissolve in 100 mL of deionized water and stirred for 30 minutes. The container was covered with aluminium foil to protect from light and store under constant conditions [12].

2.3.2 Experimental Design

Individual well in a 96-well plate contained 4×10^4 monocytes with 12×10^4 parasites (ratio 1:3). Three parallel experiments. Ag NPs at concentrations of 0.5, 1, and 2 µg/mL. Negative control without nanoparticles and incubated for 48 h at 37 °C before viability testing [13].

2.3.3 Thiazolyl Blue Tetrazolium Bromide (MTT), assay to Ag NPs

2.3.3.1 Principle

The test known as the MTT assay is a colorimetric assay commonly used to assess the activity of cellular metabolism, particularly in research about cytotoxicity and cell viability. Based on the available data, the following is a concise overview of the primary components of the test called the MTT. The MTT assay produces a violet formazan product by decreasing the yellow tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl 2,5-diphenyltetrazolium bromide). This reduction is noted in active metabolic cells, and the amount of Formosan generated is directly proportional to the quantity of cells that are viable [14].

2.3.3.2 Procedure

Following about 48 hours of incubation, 28 µL of the reagent for MTT was given to every one of the treated plates. The treated plates contained zinc nanoparticles, silver nanoparticles, and silver-zinc nanoparticles. The control plates received the same quantity of MTT reagent for all concentrations. After that, the plates spent roughly three hours in the dark. 50 µL of DMSO, which is short for dimethyl sulfoxide, was added to every well, mixed well, and left to sit in the dark for fifteen minutes to make sure the formazan crystals dissolved. The wavelength of the absorbance was quantified using a reader for microplates calibrated to 584 nm [15].

3. Results and Discussion

Using an MTT viability assay, results demonstrate how silver nanoparticles affect the macrophages of *leishmania tropica* parasites in vitro at varying doses. The highest tested amount (2.0 µg/mL) significantly reduced survival, with an average absorbance of 0.069 nm. When exposed to doses of 0.5 µg/mL and 1.0 µg/mL of silver nanoparticles, the absorbance values of the study group (0.076 and 0.075 nm, respectively) were significantly reduced. The average absorbance of the control group of 0.249 nm indicated the highest survival rate as showed in (table 1).

Table 1: Effect of Ag NPs on the viability of *leishmania* tropica promastigotes.

Ag NPs Concentration	Mean Absorbance at 584 nm	Significance (LSD test)
Control (0 µg/mL)	0.249	B
0.5 µg/mL	0.076	C
1.0 µg/mL	0.075	C
2.0 µg/mL	0.069	a
LSD	0.021	-

Significant differences between the groups were found by statistical analysis using the least significant difference (LSD) method. In comparison to the other concentrations and the control group, which had concentrations of 2.0 µg/mL,

demonstrated a significant decline in parasite viability, activity, and survival.

Given the same symbol to maintain resemblance, the two groups, with doses of 0.5 µg/mL and 1.0 µg/mL, demonstrated a substantial reduction in parasite viability when compared to the control group. These findings show that silver nanoparticles have an impact on parasite survival, with stronger antiparasitic effects at greater doses. Silver nanoparticles were not present in the control group; promastigote parasite vitality was the greatest.

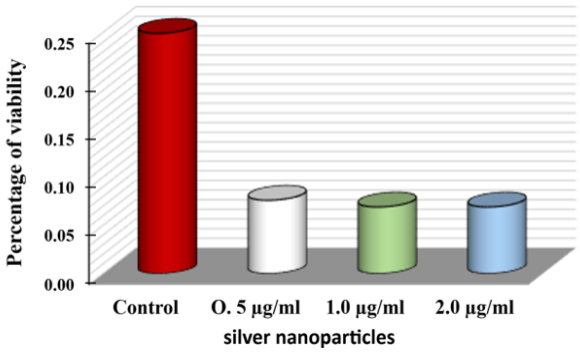

Figure 1: Graph depicting viability of Leishmania tropica promastigotes exposed to varying concentrations of Ag NPs.

Silver nanoparticles demonstrated a potent and reliable antiparasitic action, as shown in (figure 1). At 0.5 µg/mL, the vitality of *leishmania tropica promastigotes* dropped dramatically, and it remained low at 1.0 µg/mL and 2.0 µg/mL.

Silver nanoparticles caused a slow decline that varied with concentration. Aslo, at the lowest concentration, silver nanoparticles quickly reduced viability, suggesting a very efficient mode of action. These findings agree with Ray et al. [16], as well as Ahmed, and Ikram [17]. Silver nanoparticles have broad-spectrum antimicrobial activity via mechanisms like membrane disruption, reactive oxygen species (ROS) production, and interference with protein synthesis and DNA replication.

Additionally, silver nanoparticles are effective at any low concentrations, which may be because of their capacity to quickly create oxidative stress and pass through parasite membranes. From a medicinal standpoint, silver nanoparticles are interesting candidates for the development of antileishmanial medications due to their strong cytotoxic action at sub micromolar levels.

Before being used in therapeutic settings, further research is necessary to assess their cytotoxicity and selectivity in mammalian host cells. demonstrates their significant effectiveness in stimulating macrophages to eliminate parasites. In most cases, macrophages eliminate infections by producing reactive oxygen species (ROS). Using enzymatically inhibiting the production of reactive oxygen species (ROS), *leishmania* parasites may sometimes evade the immune response and continue to live within host cells. It has been shown that silver nanoparticles stimulate macrophages to produce reactive

oxygen species, to which parasites are very sensitive [18].

Additional research revealed that the production of reactive oxygen species (ROS) is the cause of this notable impact, and that silver nanoparticles function as an enzymatic source for the release of ROS [19]. Hydroxyl radicals are created when electrons expelled from the nanoparticles contact with oxygen deposited on their surfaces [20].

Apoptosis is the outcome of these radicals' damage to cellular components, including DNA and proteins [21]. A saturation threshold, where the cytotoxic response is constant despite increasing doses, may be suggested by the similar effects seen at concentrations of 0.5 and 1.0 µg/mL. The following decrease at 2.0 µg/mL suggests that higher doses consistently increase their antiparasitic effectiveness, perhaps because of increased ROS production or parasite surface nanoparticle aggregation.

It is noteworthy that no dosage assessed in this study demonstrated cytotoxicity to macrophages, indicating good therapeutic qualities. These results are consistent with other studies showing that silver nanoparticles have strong antibacterial activity and little harm to mammalian cells. As a result, silver nanoparticles represent a potential leishmaniasis treatment option or adjuvant.

Baiocco et al. [22] evaluated the *leishmaniasis*-causing effects of silver nanoparticles. The results showed that silver nanoparticles had a greater effect on leishmaniasis than pentostam, an antimony compound. Because of their large surface area per volume, silver nanoparticles are considered superior to other metal oxide nanoparticles because they are less harmful to the environment and have significant antibacterial, anticancer, and antioxidant qualities [23].

The combination of treatment, silver nanoparticles, and the loaded medication significantly slowed the development of the early stage of cutaneous leishmaniasis [24]. When given intravitreally to patients with cutaneous *leishmaniasis*, both free and loaded nanocomposites reduced the ulcer's size, increasing their efficacy.

Due to the growing demand for their special qualities in a wide range of applications, silver nanoparticles seem to have a bright future. It is anticipated that silver nanoparticles will have a significant impact on several industries, including electronics, energy, healthcare, environmental remediation, and antimicrobial applications.

Recent advances in our knowledge of silver nanoparticles' antiprotozoal properties are compiled in this publication. Number of mechanisms contribute to silver

nanoparticles' antiparasitic effectiveness, such as the rupture of parasite cell membranes, a decrease in metabolic activity, and a suppression of proper reproduction.

Leishmania infantum and *leishmania tropica* multiplication was dramatically inhibited by increasing the dosages of silver nanoparticles made from ginger extract. In *leishmania infantum* and *leishmania tropica*, flow cytometry demonstrated that, according to the MTT test, silver nanoparticles cause programmed cell death (PCD). Increased lethality to promastigotes was the outcome of higher nanoparticle concentrations, such as 80 ppm [25].

Silver nanoparticles shown generally excellent antileishmanial efficacy against Leishmania infantum and Leishmania tropica in vitro [26]. The beneficial effects of silver nanoparticles on external parasites, such those that cause *sarcoptes scabiei* in rabbits, have been shown in another research. Without having any negative effects on the animals, silver nanoparticles were successful in minimising skin lesions and enhancing general health. Moreover, modifying immunological responses, the therapy led to a significant decrease in the parasite burden and enhanced skin healing [27].

In clinical medicine, nanotherapeutic agents (NTAs) are essential if their special qualities are recognised and effectively used. The plant *Sesquium edule* was used as a reducing and capping agent in the production and characterisation of highly efficient plant-derived silver nanoparticles. In an in vitro experiment, clinical isolates of *leishmania donovani promastigote* cells were used to test silver nanoparticles' antileishmanial properties.

Antileishmanial activity was shown by silver nanoparticles made with plant extracts like ginger and *sesquium edule*, suggesting an effectiveness on par with conventional treatments like miltefosine [28]. The properties of silver nanoparticles may inhibit the growth of *leishmania* parasites. They interact with the parasites' cellular components, causing cellular death. Silver nanoparticles unique properties, such as their large surface area and ability to generate reactive oxygen species, increase their effectiveness against a variety of diseases, including leishmania species [29].

ROS may result in oxidative stress, DNA damage, and eventually parasite death [30]. Plasmodium, the parasite that causes malaria, is one example of a protozoan that silver nanoparticles have shown to be effective against. These parasites' ability to survive may be severely hampered by the emitted silver ions [31]. When compared to traditional treatments that require significant financial commitments, novel

therapies especially those that use nanotechnology can be verified [32].

Silver nanoparticle exposure may alter parasite gene expression, upsetting vital cellular pathways and functions. This involves breaking down proteins, perhaps harming DNA, and triggering processes like detoxification. It has been shown that silver nanoparticles disrupt the development and reproduction of parasites, influencing both the life cycle phases and reproduction [33].

Silver nanoparticles could pierce parasite cell membranes and harm their structural integrity. Cell death may result from this disturbance, which may also cause cell contents to seep out. However, by attaching to ribosomes or other elements involved in protein synthesis, silver nanoparticles may interfere with the creation of proteins necessary for parasite survival and reproduction [34].

Silver nanoparticles may assist parasites live at first by activating their detoxification pathways, but over time, this may cause metabolic depletion [35]. The research showed that 48 hours after treatment, non-toxic quantities of silver nanoparticles might boost the inhibitory effects of *leishmania tropica amastigotes* and promastigotes in vitro and activate macrophages.

Based on current nanotechnology, these discoveries might aid in the creation of safe, non-toxic, and potent anti-*leishmanial* medications to combat *leishmaniasis* [36]. Highly effective silver nanoparticles synthesized from the plant *Schidium edule* were produced and characterized. In addition, electrochemical techniques were used to verify the synthesis of silver nanoparticles. Clinical isolates of *leishmania donovani promastigote* cells [37], were used in an in vitro experiment to evaluate the antileishmanial effectiveness of silver nanoparticles. Silver nanoparticles did not significantly harm normal mammalian cells, according to statistical analysis of the data [28, 37].

4. Conclusion

Silver nanoparticles are more effective and efficacious in eliminating cutaneous *leishmaniasis* at the promastigote stage, according to this study. The results pertaining to silver nanoparticles were widely accepted. Significant antibacterial activity has been demonstrated by silver nanoparticles against a variety of diseases, including the *leishmania* parasites that cause *leishmaniasis*. Their ability to generate reactive oxygen species (ROS) and release silver ions, which can disrupt cellular processes and cause parasite cell death, is thought to be the reason for their effectiveness. The study shows silver nanoparticles work to lower *leishmania*

tropica parasite survival in vitro. At the measured concentrations, silver nanoparticles are non-toxic to host macrophages but directly harm cells by circumventing the parasite's defence systems.

5. References

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