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Cytotoxicity Effect Of Combined Silver And Zinc Oxide Nanoparticles (Agnps + Zno Nps) On Phagocytic And Cytotoxic Activity Of Macrophages To Cutaneous Leishmania Parasite In Vitro

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Abstract

This study evaluated the ability of zinc nanoparticles (ZnO NPs) and silver nanoparticles (AgNPs) combined to stimulate macrophages to produce reactive oxygen species (ROS) capable of destroying Leishmania tropica parasites. This study used quantitative bioassays via MTT assays. The primary objective was to explore the efficacy of the nanoparticles. .In order to assess the possible additive or synergistic effects of mixing these nanoparticles, four duplicates of each concentration (0.5, 1.0, and 2.0 µg/ml) were employed. Statistical validation used the least significant difference (LSD) to validate the test results, revealing significant differences between treatments, clearly demonstrating their significant antiparasitic efficacy

INTRODUCTION

Numerous species of Leishmania, flagellated protozoa (kinetoplastids) that are members of the phylum Euglenozoa and kingdom Prostista, are responsible for leishmaniasis, a disease that can seriously harm both people and animals (Roberts, 2006). The parasite behind Indian kala-azar ("Dum-Dum fever") was first identified by Leishman in 1900 from a post-mortem case, though details weren't published until May 1903. Shortly after, in July 1903, Donovan discovered similar organisms in Madras cases, and Rogers successfully cultivated the parasite in July 1904. The disease is believed to have originated in Assam and is also found in Madras, Ceylon, Burma, Indo-China, China, and Syria. The causative species of leishmaniasis were discovered in the early 20th century, and the disease has historically been divided into three categories: mucocutaneous (tegumentary) leishmaniasis (L. braziliensis), cutaneous leishmaniasis (Oriental sore, L. tropica), and visceral leishmaniasis (kala-azar, caused by L. donovani). Between 1918 and 1923, kala-azar killed almost 200,000 people in Assam and the Brahmaputra valley, highlighting the disease's lethality. In 1944, there was yet another outbreak. Kala azar was once known as Assam fever. (Elias and others, 1989). Research in Iraq identified the parasite causing ulcerative skin lesions in Baghdad (Wenyon, 1911). Later studies documented the prevalence of visceral leishmaniasis (VL) across most of Iraq, with the exception of the southeastern marsh regions (Rahim, 1967), and confirmed its endemic status in Baghdad and surrounding areas (Nouri and Al-Jebori, 1973). Every year, leishmaniasis is acknowledged as a major tropical illness worldwide, and the World Health Organization/Tropical illness Research (WHO/TDR) regularly ranks it as one of the most significant diseases (Herwaldt, 1999; Desjeux, 2001).

Materials and Methods

Parasite culture

Isolates of Leishmania tropica promastigotes were obtained from a private laboratory where continuous .subculture procedures were performed under sterile conditions Following material preparation, 100 ml of supplemented RPMI 1640 medium (10% foetal calf serum, 1% gentamicin) was added to culture flasks. Then, 1 ml of parasite suspension was carefully added to each prepared flask. The culture flasks were tightly sealed

and then incubated at 25°C. Parasite growth was monitored daily using a light microscope to observe .morphological changes and ensure the viability of the cultures

Culture of monocytes

.A similar volume of PBS was used to dilute the venous blood sampFollowing dilution, the blood sample was combined with Ficoll-Paque in a centrifuge tube and centrifuged for 30-40 minutes at 400 g and room temperature.

Monocytes formed a noticeable white layer between the plasma and Ficoll-Paque after centrifugation. The layer of monocytes was aspirated and transferred to a new tube. The monocytes were centrifuged 300 times for 10 minutes to wash withPBS After washing, the monocytes were transferred to RPMI 1640 culture media supplemented with 50 µg/ml gentamicin and 10% FBS, and they were then incubated at 26°C.

Preparation of (Ag NPs) and (Zn NPs) concentration

First, silver nanoparticles were obtained from a manograph, and a solution was made by dissolving 0.169 g in 100 ml of deionized water. 0.4 g of zinc nanoparticles were dissolved in 100 ml of deionised water to create a separate solution, which was then continuously stirred magnetically for 30 minutes to guarantee uniform particle dispersion. This zinc nanoparticle solution was stored in a glass vial, protected by aluminum foil, under controlled laboratory conditions until required. Three different concentrations of these nanoparticles (0.5, 1.0, and 2.0 µg/ml) were then made ready for usage.

Experimental Design

To assess the impact of different zinc and silver nanoparticle concentrations on protozoa and monocytic cells, L. tropica (12 × 10^4 parasites per well) was introduced to protozoa (4 × 10^4 cells per well). First, each well received 100 µL of protozoan culture. After that, 200 µL of protozoan parasites were added, bringing the protozoa to parasite ratio to 1:3. 50 µL of serially diluted zinc and silver nanoparticles (at values of 0.5, 1, and 2 µg/ml) were added following an incubation period. As a control, one well was not injected and was incubated for 48 hours at 37°C.

Basic MTT Test Kit (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)

Widely employed in cytotoxicity and cell viability studies, the MTT test is a color-based assay for assessing cellular metabolic activity. Its main components are briefly outlined here.
The foundation of the test is metabolically active cells' conversion of the yellow MTT salt (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) to purple formazan; the amount of formazan generated is directly correlated with the number of live cells.

Results and Discussion

The MTT viability test was used to evaluate the efficacy of a formulation of zinc oxide nanoparticles (ZnO NPs) and silver (AgNPs) against Leishmania tropica promastigotes. Four experimental groups participated in the study: three treatment groups were exposed to the combined nanoparticles at doses of 0.5 µg/mL, 1.0 µg/mL, and 2.0 µg/mL, while a control group received no nanoparticles (0.0 µg/mL). The control group displayed the highest mean viability (0.342). Promastigotes treated with the nanoparticle combinations :showed reduced viability values as follows

The control group displayed the highest mean viability (0.342). Promastigotes treated with the nanoparticle combinations showed reduced viability values as follows 0.157 at 0.5 µg/ml
at 1.0 µg/mL، 0.080 at 2.0 µg/ml 0.141
.These results reflect a clear trend of decreasing parasite viability with increasing nanoparticle concentration

:According to LSD statistical analysis (LSD = 0.110)

Table 1: Effect of AgNPs + ZnO NPs on the viability of *Leishmania tropica* promastigotes

AgNPs + ZnO NPs Concentration	Mean Absorbance at 584 nm	Significance (LSD test)
Control (0 µg/ml)	0.342	a
0.5 µg/ml	0.157	b
1.0 µg/ml	0.141	b
2.0 µg/ml	0.080	b
LSD	0.110	-

Significant differences (P < 0.05) exist across means that do not share a letter.

.The control group was assigned the letter "a", indicating a significant difference from all treated groups .All treated groups were marked "b", suggesting no significant difference among the tested doses This indicates a strong antiparasitic effect of the combination, even at lower doses, with a possible .saturation effect beyond 0.5 µg/ml

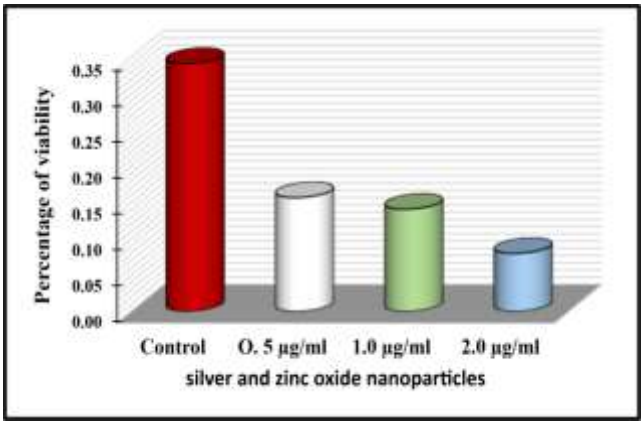

Figure 1: Graph showing viability of *Leishmania tropica* promastigotes exposed to AgNPs + ZnO NPs at different concentrations.

The vitality of Leishmania tropica promastigotes was dramatically reduced by the combination of silver and zinc oxide nanoparticles (AgNPs + ZnO NPs), as shown in Figure 3.3. The impact grew with increasing dose. The control group showed the highest viability, while 0.5 µg/ml treatment caused a noticeable drop. Further reductions were seen at 1.0 µg/ml and were most pronounced at 2.0 µg/ml, indicating enhanced .antiparasitic efficacy with increasing concentration This combined formulation appears to harness the complementary mechanisms of both nanoparticles. Silver nanoparticles are known to disrupt membranes and interfere with DNA and protein synthesis (Rai et al., 2012), while ZnO NPs generate reactive oxygen .species and release zinc ions that impair parasite metabolism (Sharma et al., 2012; Sirelkhatim et al., 2015) The observed synergy may reflect overlapping stress pathways, although the effect is not dramatically .higher than AgNPs alone, suggesting additive rather than fully synergistic action

These results suggest combining
metal nanoparticles could be a promising approach to enhancing antileishmanial activity while potentially lowering individual nanoparticle doses to reduce toxicity. Further mechanistic and in vivo studies are .necessary to evaluate the' safety, pharmacokinetics, and therapeutic advantage of such nanocomposites

Leishmania parasites inhibit the production of ROS through enzymatic pathways, resulting in the survival of Leishmania within macrophages as host cells (Mehta and Shaha 2006). ROS-scavenging nanoparticles .,may overcome the inhibition of ROS production in macrophages by Leishmania isolates (Chatterjee et al Most often, the harmful effects of ROS may manifest through the oxidation of polyunsaturated fatty .(2011 acids in lipids, the oxidation of amino acids in proteins, and DNA damage (Limbach et al,. 2007). Silver nanoparticles demonstrated a certain ability to generate high amounts of reactive oxygen species (ROS) in macrophages, the host cells of Leishmania parasites. ROS can cause oxidative stress, DNA damage, and ultimately parasite death(Rawa and Entsar,. 2019)

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