



Greener Synthesis of Rod Shaped Zinc Oxide NPs Using *Lilium ledebourii* Tuber and Evaluation of Their Leishmanicidal Activity

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Background: NPs (NPs) with unique chemical and physical properties can be used for therapeutic purposes because of their strong antimicrobial activities. NPs have been used as antimicrobial agents to inhibit microbial growth.

Objectives: In view of the strong antimicrobial activity of NPs, the biogenic synthesis and leishmanicidal activity of rod-shaped zinc oxide (R-ZnO) NPs was explored using *Lilium ledebourii* tuber extract.

Materials and Methods: The ensuing NPs are characterized by UV-visible spectroscopy, X-ray diffraction and transmission electron microscopy and their leishmanicidal activity evaluated against the *Leishmania major* (*L. major*) by MTT assay.

Results: The R-ZnO NPs displayed excellent leishmanicidal activity against the *L. major* as they significantly inhibited the amastigotes. The IC₅₀ values of R-ZnO NPs being ~ 0.001 mg.mL⁻¹. R-ZnO NPs can inhibit *L. major* growth in a dose-dependent manner under *in vitro* conditions.

Conclusion: A simple, low-cost feasible and eco-friendly procedure was developed for biosynthesis of R-ZnO NPs using natural bioresource that can inhibit human parasite cells growth in a dose-dependent manner under *in vitro* conditions.

Keywords: Biosynthesis, Leishmanicidal, NPs, Rod-shaped, Zinc Oxide.

1. Background

Leishmaniasis is a disease caused by *Leishmania* species with the incidence rate of about two million cases annually (1). Currently, the increase in international travel and environmental amendments caused by war in some regions has created favorable conditions for the propagation of the parasite vectors; thus, there has been a significant increase in the incidence of leishmaniasis (2). Poverty contributes to the risk of leishmaniasis and so does the open sewerage and lack of waste management which can increase sandfly breeding sites of *Leishmania* vectors, as well as their access to human bodies. About 70 animal species have been found to act as natural reservoir hosts for these parasites (3). The first choice for the treatment of leishmaniasis is pentavalent antimoniate (Pa). But the Pa—because of its side effects, the emergence of resistance, and parenteral application is no longer a sufficient treatment. Therefore,

it is essential to find new drugs with different mechanisms of greater potency (4).

Nanoparticles (NPs) can be used for therapeutic applications because of their strong antimicrobial activities (5-7). Such as in drug delivery, microbiology, biotechnology, and biochemistry (8-15). The use of metal NPs such as copper, platinum, titanium, gold, selenium, silver, zinc oxide, and palladium NPs against many bacteria, viruses, and fungal pathogens has been reported (16-19), but the use of NPs against protozoan parasites is rather limited. Among different types of NPs, the zinc oxide (ZnO) NPs have attracted scientific attention because of their safety and high antimicrobial activity (20, 21); they are recognized to be safe by the U.S. Food and Drug Administration (FDA). The traditional NPs synthesized by chemical methods leads to the adsorption of toxic chemical compounds onto the surface of synthesized NPs which may have adverse

effects in medicine (22, 23). Green synthesis methods using bioresources such as plants, fungi, or bacteria for the synthesis of biogenic NPs represent alternatives to conventional chemical synthesis methods (24-29).

The present study aims to evaluate the leishmanicidal efficiency of rod-shaped ZnO (R-ZnO) NPs on *Leishmania major* cultures.

2. Objectives

The main aim of this study was to evaluate green synthesis of parasitological zinc oxide NPs using *Lilium ledebourii* tuber. A greener synthesis of rod shape zinc oxide (R-ZnO) NPs were studied using *L. ledebourii* tuber as a novel bioresource and their leishmanicidal activity has been studied against *L. major*.

3. Materials and Methods

3.1. Materials

All the reagents and chemicals used in the experiments were purchased from Merck, Germany.

3.2. Synthesis of Rod Shaped ZnO NPs

The *L. ledebourii* tuber were washed thoroughly with sterile distilled water and dried, then 10 g of *Lilium* tuber was ground into a powder. The powder was added to 200 mL of deionized double-distilled water, heated at 80 °C, and then filtered. To conduct green synthesis of R-ZnO NPs, 20 mL of the obtained extract was added to the 100 mL of zinc acetate dehydrate solution and stirred at ~80 °C for 1 hr. The reaction mixture (extract + zinc acetate dehydrate) was incubated at 80 °C for 2 hr and calcined at 300 °C for 1 hr.

3.3. Characterization of ZnO NPs

The synthesized ZnO NPs were studied using a UV-visible spectrophotometer (Analytik Jena; Germany). XRD analysis was performed to determine the formation of ZnO crystals. The resulting powder was analyzed using an X-ray diffractometer (PANalytical X'Pert PRO; The Netherlands) at 2θ . The shape, size and size distribution of nanoparticle were investigated by TEM (30).

3.4. Leishmanicidal Assay

The *L. major* MRHO/IR/75/ER standard strain was cultured in RPMI1640 at 25 °C supplemented with 15% heat-inactivated FBS, streptomycin (200 mg.mL⁻¹), and penicillin (200 IU.mg.mL⁻¹). The stationary growth phase of promastigotes was added to the macrophages to generate a parasite/macrophage ratio of 10:1. It was then incubated at 37 °C in 5% CO₂ for 24 hr (31). The macrophages containing antiamastigote were treated with various concentrations of 0–500 mg.mL⁻¹ R-ZnO NPs (32).

3.5. Statistical Analysis

The differences between the groups were determined using one-way analysis of variance (ANOVA) to analyse the obtained results. A *p*-value < 0.05 was considered significant.

4. Results

4.1. Biosynthesis and Characterization of ZnO NPs

The UV-Vis spectrum at 350–370 nm wavelengths (**Fig. 1**) shows the synthesis of R-ZnO NPs to be consistent with previous findings (33).

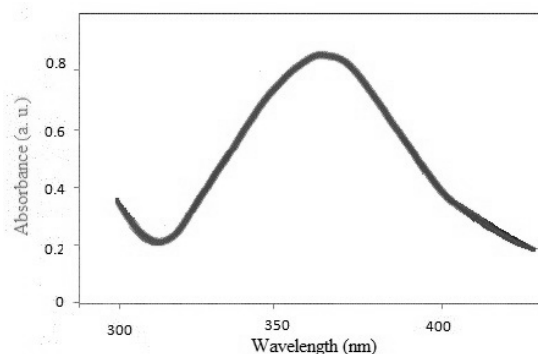


Fig. 1. UV-visible spectroscopy of biosynthesized ZnO nanoparticles

The XRD pattern shows dispersion peaks at 31, 35, 37, 48, 57, 62, 66, 68, and 69, thus confirming the presence of ZnO NPs in the sample (**Fig. 2**), which corroborated previous with previous findings (33, 34).

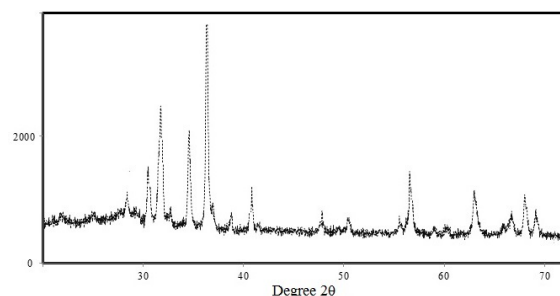


Fig. 2. XRD pattern of biosynthesized R-ZnO nanoparticles.

TEM images confirm that biogenic ZnO NPs have rod-shaped morphology; however, spherical NPs were observed in the TEM images. R-ZnO NPs are below 100 nm in size (**Fig. 3**).

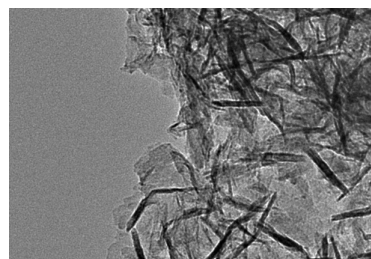


Fig. 3. TEM image of biosynthesized R-ZnO NPs nanoparticles.

4.2. Antiamastigote Assay

The R-ZnO NPs were found to inhibit the multiplication rate of amastigotes in a dose-dependent manner (Fig. 4). The IC_{50} values were about 10 mg.mL^{-1} for both R-ZnO NPs and Meglumine antimoniate (glucantime) as positive control. The concentration of 0.5 mg.mL^{-1} of biogenic R-ZnO NPs revealed a higher toxicity effect on *L. major* (amastigotes). The results disclose that by increasing the concentration of R-ZnO NPs, the viability of the tested parasites significantly decreases.

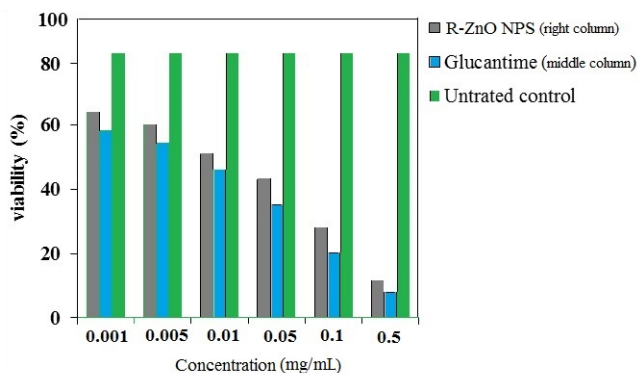


Fig. 4. The effect of R-ZnO NPs and Glucantime on viability of *Leishmania major* (amastigotes).

5. Discussion

In this study, for the first time, the rod shaped ZnO NPs were biosynthesized as a safe therapeutic nanomaterial. A novel protocol for the greener production of the rod shaped ZnO NPs is presented in this study. The traditional NPs synthesized by chemical methods leads to the adsorption of toxic chemical compounds onto the surface of synthesized NPs which may have adverse effects in medicine. The NPs that are produced by plant extract have lower environmental risk due to lack of harsh chemicals often in their synthesis process. Therefore, they can be applied in medical programs such as drug delivery. The salient advantages of producing plant NPs via greener methods such as using bacterial or fungal extracts is the safety and high availability of medicinal plants that are more reliable and healthier than bacterial or fungal extracts mediated for the production of NPs. Green synthesis methods using bioresources such as plants, fungi, or bacteria for the synthesis of biogenic NPs represent alternatives to conventional chemical synthesis methods

The use of factors present in the plant and fungus residue not only are responsible in the synthesis of NPs, but by stacking around the NPs, they cause stability and prevent their agglomeration. Plants, in addition to being non-toxic, have different types of metabolites, which can be effective in the synthesis of NPs which

can include terpenoids, flavonoids, carbonyls, amides, and carboxylic acids, which directly contribute to the formation of NPs.

The result of a study conducted against *L. major* showed that rod shaped ZnO NPs have good leishmanicidal activity against *L. major* and this outcome could help in the development of formulations as efficient means to synthesize R-ZnO NPs from natural products in our fight against the resistant microorganisms (35, 36).

In our study, the rod shaped ZnO NPs displayed strong leishmanicidal efficiency (IC_{50} about 0.012 mg.mL^{-1}). There are no reports on the leishmanicidal activity of biogenic rod shaped ZnO NPs nor on the cytotoxic effect of chemically synthesized Rod shaped ZnO NPs on living cells. The study by Delavari *et al.* 2014 (37) have reported leishmanicidal activity of chemically synthesized spherical shaped ZnO NPs on *L. major*, the IC_{50} ZnO NPs being $0.0378 \text{ mg.mL}^{-1}$ on promastigotes of *L. major*. Also Sumaira *et al.* (38) have reported leishmanicidal activity of spherical greener synthesized ZnO NPs, the IC_{50} ZnO NPs being 0.25 mg.mL^{-1} against *L. tropica*. But in our present study, the biogenic greener synthesized NPs rod shaped ZnO NPs displayed much stronger leishmanicidal activity (IC_{50} about 0.012 mg.mL^{-1}); they are more effective in leishmanicidal activity compared to chemically assembled spherical shape ZnO NPs or greener spherical shaped ZnO nanoarticles. Additionally, biogenic R-ZnO NPs can also be used *in vivo*.

6. Conclusion

These results show that it is possible to prepare a safe and ecofriendly synthesized NPs with leishmanicidal potential. The greener synthesized rod shaped ZnO NPs, displayed stronger leishmanicidal activity (IC_{50} about 0.012 mg.mL^{-1}); compared to chemically assembled spherical shape ZnO NPs and greener spherical shaped ZnO NPs (37).

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Competing interests

The authors confirm that this article content has no competing interests.

Authors' Contribution

All authors have participated in the manuscript preparation.

Financial Disclosure

There is no conflict of interest

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