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Isatis tinctoria mediated synthesis of amphotericin B-bound silver nanoparticles with enhanced photoinduced antileishmanial activity: A novel green approach

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ABSTRACT

After malaria, Leishmaniasis is the most prevalent infectious disease in terms of fatality and geographical distribution. The availability of a limited number of antileishmanial agents, emerging resistance to the available drugs, and the high cost of treatment complicate the treatment of leishmaniasis. To overcome these issues, critical research for new therapeutic agents with enhanced antileishmanial potential and low treatment cost is needed. In this contribution, we developed a green protocol to prepare biogenic silver nanoparticles (AgNPs) and amphotericin B-bound biogenic silver nanoparticles (AmB-AgNPs). Phytochemicals from the aqueous extract of *Isatis tinctoria* were used as reducing and capping agents to prepare silver nanoparticles. Amphotericin B was successfully adsorbed on the surface of biogenic silver nanoparticles. The prepared nanoparticles were characterized by various analytical techniques. UV–Visible spectroscopy was employed to detect the characteristic localized surface plasmon resonance peaks (LSPR) for the prepared nanoparticles. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) studies revealed the formation of spherical silver nanoparticles with an average particle size of 10–20 nm. The cubic crystalline structure of the prepared nanoparticles was confirmed by X-ray diffraction (XRD) study. FTIR spectroscopic analysis revealed that plant polyphenolic compounds are mainly involved in metal reduction and capping. Under visible light irradiation, biogenic silver nanoparticles exhibited significant activity against *Leishmania tropica* with an IC_{50} value of 4.2 μg/mL. The leishmanicidal activity of these nanoparticles was considerably enhanced by conjugation with amphotericin B (IC_{50} = 2.43 μg/mL). In conclusion, the findings of this study reveal that adsorption of amphotericin B, an antileishmanial drug, to biogenic silver nanoparticles, could be a safe, more effective and economic alternative to the available antileishmanial strategies.

1. Introduction

Leishmaniasis, an uncontrolled and emerging disease, constitutes a major health problem worldwide, causing the significant rate of morbidity and mortality in Asia, Africa, and the Americas. The disease is characterized by various clinical forms (cutaneous, mucocutaneous, and visceral) and is caused by protozoa of the genus *Leishmania* [1]. The disease is prevalent in more than 80 countries affecting approximately 12 million people around the globe. The worldwide annual incidence of visceral and cutaneous leishmaniasis is estimated at 0.5 and 1.5 million respectively and it is believed that more than 3 million people are at risk [2]. There are several species of *Leishmania* causing a variety of clinical symptoms ranging from self-healing cutaneous caused by *Leishmania* major to life-threatening visceral form caused by *Leishmania donovani*. The current antileishmanial drugs are very expensive and associated with severe side effects. In addition, several *Leishmania* species have shown resistance to the available antileishmanial therapies [3,4]. Therefore, an intense need exists to develop new therapeutic strategies that are more effective, less toxic and within the reach of the poor community most affected by the disease.

In the enduring search for better leishmanicidal agents, nanobiotechnology is gaining an immense interest in the development of nanoscale materials that have attracted significant attention in the field of medicine. These nanomaterials have unique physiochemical properties that find applications in various disciplines including catalysis, cosmetics, and drug delivery systems [5,6,7]. Among the nanoscale materials, silver nanoparticles are of significant importance as they possess a broad spectrum of antimicrobial properties and could be an alternative and more effective agent in the treatment of emerging microbial

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resistance. Silver nanoparticles exert a complex mechanism of antimicrobial action, inhibiting metabolically critical enzymes, disrupt cell membrane, generating reactive oxygen species (ROS) and interfere with microbial DNA replication [8,9,10]. Furthermore, conjugation of these nanoparticles with amphotericin B (AmB), an antileishmanial drug, would boost the drug efficacy against the emerging leishmanial resistance. Amphotericin B is an effective agent against leishmania; however, severe side effects and cost of this drug limit its application. In its free form, amphotericin B is assembled into higher order molecular forms which bestow it with its toxic property. Conjugation of AmB to metal nanoparticles would minimize the severe toxicity associated with this drug. In addition, the antimicrobial property of silver nanoparticles would enhance the antileishmanial activity of conjugated AmB (synergism).

Silver nanoparticles have been prepared both by physical and chemical methods [11,12,13]. However, all those synthesis procedures are associated with several drawbacks such as the use of expensive and hazardous chemicals and are, therefore, not friendly both from the economical and environmental points of view. Hence, new strategies are needed to prepare metal nanoparticles by a simple and green procedure. Plant kingdom presents the most versatile source of green chemicals that could be successfully utilized for the large scale synthesis of metal nanoparticles. The aqueous extracts of medicinal plants contain various therapeutic agents that can be used as reducing and stabilizing agents for the synthesis of metal nanoparticles [14,15,16,17,18]. In addition, the bioactive capping molecules from the plants would enhance the antimicrobial property of metal nanoparticles and will remain active even after the depletion of capped silver.

Isatis tinctoria is a well-known medicinal herb used in Chinese folk and modern medicine for the treatment of gastroenteritis, encephalitis, and upper respiratory tract infections [19]. The aqueous root extract of this plant has been reported for its strong antimicrobial, antiviral and anti-parasitic activities [20,21]. Phytochemical investigation of *I. tinctoria* leaf extracts has revealed the presence of various anti-inflammatory compounds such as tryptanthrin, ferulic acid and sinapic acid [21]. The root extract of this plant contains a potent anticancer agent (indirubin) which has been reported as a strong anticancer compound for the treatment of chronic myelocytic leukemia and solid tumors [22]. Based on its phytochemistry and biomedical applications, *I. tinctoria* was selected for the synthesis of bioactive silver nanoparticles.

In this contribution, we report, for the first time, the *I. tinctoria* mediated green synthesis of silver nanoparticles (AgNPs) and amphotericin B bound green synthesized silver nanoparticles (AmB-AgNPs). The prepared nanoparticles were fully characterized using various analytical techniques. The leishmanicidal property of these nanoparticles was tested against *Leishmania tropica* in different conditions (dark and visible light).

2. Material and Methods

2.1. Materials

I. tinctoria (Chinese medicinal plant) was purchased from a local market in Beijing, China. Silver nitrate ($AgNO_3$) and amphotericin B were purchased from Beijing Chemical Works and Sigma-Aldrich respectively. All the chemicals used in this study were of analytical grade. Millipore (18.2 MΩ cm) water was used as a solvent throughout the experiments.

2.2. Preparation of Plant Extract

The dried plant material (powder) was thoroughly washed with distilled water to remove any dust particles. 20 g of the dust-free plant material was extracted with 200 mL Millipore water. The biomass was initially heated at 60 °C for 30 min and then magnetically stirred (300 rpm) for 2 h at room temperature (26 °C). Finally, the extracted biomass was filtered (Whatman No. 1) and the clear supernatant obtained was stored at 4 °C for further use.

2.3. Synthesis of Silver Nanoparticles

For the synthesis of silver nanoparticles (AgNPs), different concentrations of plant extract (mL) and silver nitrate solutions were used to get the optimized product. In a typical procedure, 10 mL of plant extract was mixed with 50 mL of silver nitrate solution (3 mM) and the progress of nanoparticle synthesis was monitored by the color change from light yellow to deep brown. UV–visible spectroscopy was used to detect the appearance of localized surface plasmon resonance (LSPR) peaks for silver nanoparticles. When the LSPR peaks reached its maximum saturation, the reaction mixture was centrifuged at 12,000 rpm for 10 min. The pellet obtained was washed twice with Millipore water to remove any un-reacted silver and plant material. Finally, the pure product (AgNPs) was freeze dried and stored at room temperature (26 °C) for further use.

2.4. Preparation of Amphotericin B-bound Silver Nanoparticles

In order to prepare amphotericin B bound silver nanoparticles (AmB-AgNPs), 5 mL of amphotericin B solution (200 μg/mL) was added to a 50 mL suspension of phytosynthesized silver nanoparticles. The reaction mixture was magnetically stirred (300 rpm) for 3 h at room temperature (26 °C). UV–visible spectroscopy was used to detect the position of LSPR peaks for amphotericin B-adsorbed silver nanoparticles. The reaction mixture was centrifuged at 12,000 rpm for 10 min and the pellet (AmB-AgNPs) obtained was washed twice with distilled water. Finally, the prepared nanoparticles were freeze-dried and were stored at room temperature (26 °C) for further study.

2.5. Characterization of Nanoparticles

Both the prepared nanoparticles were fully characterized by various analytical techniques. The appearance of specific LSPR peaks for the prepared nanoparticles was monitored by UV–Vis spectroscopy (Spectrophotometer Shimadzu, 2450). The elemental composition and crystalline nature of silver nanoparticles were determined by X-ray diffraction (Powder X-ray—D8 advanced diffractometer, BRUKER) and Energy dispersive X-ray (JEOL-JEM 3010) studies respectively. Particle size and morphology were studied using transmission electron microscopy (FEI—Tecnai G^2 20 transmission electron microscope) and dynamic light scattering (HORIBA Zetasizer SZ100) studies. The possible biomolecules that are involved in the synthesis of silver nanoparticles were identified by FTIR spectroscopy (BRUKER 3000 Hyperion Microscope).

2.6. Quantification of Silver Ions

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, PerkinElmer Optima 8000) was used to determine the concentration of released silver ions from the prepared silver nanoparticles. In preparation for ICP analysis, the sample (1 mg) was digested in a solution of concentrated HCl and HNO_3 (3:1 v/v) and sonicated for 20 min. The resulting solution was then dispersed into 100 mL of deionized water in a volumetric flask. The concentration of released silver ions was calculated from $AgNO_3$ standard graph (1–50 μg/mL) plotted based on Inductively Coupled Plasma Atomic Emission Spectrometry [23].

2.7. Antileishmanial Activity

The antileishmanial activity of the prepared nanoparticles was tested against *L. tropica*. The protozoan was cultured in medium 199 containing 10% inactivated fetal bovine serum (FBS). The antileishmanial activity of the prepared silver nanoparticles was