Microbial Pathogenesis 91 (2016) 129–135

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Antibacterial, antibiofilm and cytotoxic effects of *Nigella sativa* essential oil coated gold nanoparticles

Sivalingam Manju [a], Balasubramanian Malaikozhundan [a], Sekar Vijayakumar [a], Sathappan Shanthi [a], Ameeramja Jaishabanu [b], Perumal Ekambaram [b], Baskaralingam Vaseeharan [a, *]

[a] *Crustacean Molecular Biology and Genomics Lab, Department of Animal Health and Management, 4th Floor, Science Block, Alagappa University, Karaikudi 630 003, Tamil Nadu, India*
[b] *Molecular Toxicology Laboratory, Department of Biotechnology, Bharathiar University, Coimbatore 641 046, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 3 September 2015
Received in revised form
15 November 2015
Accepted 19 November 2015
Available online 15 December 2015

Keywords:
Essential oil
AuNPs
Antibacterial
Antibiofilm
Transmission electron microscopy (TEM)
Infrared spectroscopy
Cytotoxicity
Lung cancer cells

ABSTRACT

This study reports the biological synthesis of gold nanoparticles using essential oil of *Nigella sativa* (*Ns*EO-AuNPs). The synthesized *Ns*EO-AuNPs were characterized by UV–visible spectra, X-ray diffraction (XRD), FTIR and Transmission electron microscopy (TEM). UV–vis spectra of *Ns*EO-AuNPs showed strong absorption peak at 540 nm. The X-ray diffraction analysis revealed crystalline nature of nanoparticle with distinctive facets (111, 200, 220 and 311 planes) of *Ns*EO-AuNPs. The FTIR spectra recorded peaks at 3388, 2842, 1685, 1607, 1391 and 1018 cm^{-1}. TEM studies showed the spherical shape of nanoparticles and the particle size ranges between 15.6 and 28.4 nm. The antibacterial activity of *Ns*EO-AuNPs was greater against Gram positive *Staphylococcus aureus* MTCC 9542 (16 mm) than Gram negative *Vibrio harveyi* MTCC 7771 (5 mm) at the concentration of 10 $\mu g\ ml^{-1}$. *Ns*EO-AuNPs effectively inhibited the biofilm formation of *S. aureus* and *V. harveyi* by decreasing the hydrophobicity index (78% and 46% respectively). The *in-vitro* anti-lung cancer activity confirmed by MTT assay on the cell line of A549 carcinoma cells showed IC_{50} values of bulk Au at 87.2 $\mu g\ ml^{-1}$, *N. sativa* essential oil at 64.15 $\mu g\ ml^{-1}$ and *Ns*EO-AuNPs at 28.37 $\mu g\ ml^{-1}$. The IC50 value showed that *Ns*EO-AuNPs was highly effective in inhibiting the A549 lung cancer cells compared to bulk Au and *N. sativa* essential oil.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

In recent years, biosynthesis of gold nanoparticles (AuNPs) have gained much attention and emerged to be a thrust area of research in the field of nanotechnology. Gold and zinc oxide nanoparticles has remarkable novel properties such as intense plasmon resonance, electrical, magnetic, thermal conductivity, chemical and biostability, catalytic activity, anti-bacterial activity, anti-HIV activity, anti-angiogenesis activity, anti-malarial agent and anti-arthritic activity over last few decades [1–3]. Recently, *in vitro* studies showed that AuNPs do not cause cytotoxicity in human cell and therefore, AuNPs have received tremendous interest for modern biomedical sciences, including cancer photodiagnostics, photothermal therapy, biolabeling, nanodiagnostics, drug delivery, gene delivery, immunochromatographic identification of pathogens in clinical specimen [1,2].

The biofilms are surface attached microbial communities embedded in their own microbial-originated matrix of protective and adhesive extracellular polymeric substances (EPSs), mainly polysaccharides, lipids and proteins resistant to antimicrobials [4]. The upcoming approach towards control of biofilms formation involves nanomaterials, which inhibit bacterial adhesion and biofilm formation. NPs with biocidal properties are emerging as new and promising antimicrobial agents as bacteria are less likely to develop resistance against metal NPs than conventional antibiotics [5,6].

Ionic forms of gold shown to have cytotoxicity on various cell types and adverse effects on red blood cells. It has also been reported that AuNPs synthesized by physical and chemical methods aggregates in physiological conditions hindering its *invivo* applications [7,8]. Currently, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. The biosynthesis of gold nanoparticles in plants such

* Corresponding author.
E-mail address: vaseeharanb@gmail.com (B. Vaseeharan).

http://dx.doi.org/10.1016/j.micpath.2015.11.021
0882-4010/© 2015 Elsevier Ltd. All rights reserved.

as *Terminalia catappa* [9] tea [10] lemongrass [11] has been reported. The essential oil (EOs) of plants is becoming increasingly popular as natural antimicrobial agents to be used for a wide variety of purposes including food preservation, complementary medicine and natural therapeutics, whereas the potential use of these oils as natural antimicrobial agents has been less explored [12,13]. Since ancient times, the seeds, oils and extracts of *Nigella sativa* have been used as an anticancer agent by Unani, Ayurveda and the Chinese system of medicine. It is also known that nanoparticle possess broad spectrum of activity and currently research on nanoparticle synthesis mainly focused towards its biomedical applications such as treatment of infectious diseases and cancer. Therefore, in the present study, for the first time, we report the *in vitro* cytotoxicity of *Nigella sativa* essential oil coated gold nanoparticles (*Ns*EO-AuNPs) against human lung cancer cells, A549. The antibacterial and antibiofilm potential of *Ns*EO-AuNPs were tested against Gram positive and Gram negative bacteria.

2. Materials and methods

2.1. Collection of N. sativa seeds and essential oil extraction

Fresh seeds of *N. sativa* were collected from in and around the regions of Karaikudi, Tamil Nadu, India. The extraction of essential oil was carried out by following the method of Shariff [14]. Briefly, 200 g of air dried seeds of *N. sativa* was soaked in 200 ml of water for 12 h and was subjected to hydro distillation in a Clevenger-type apparatus. The mixture was heated to form the vapor that contained essential oils. The vapor condensed in the condenser and dropped into the collecting tube. The extraction was carried out for 12 h. After extraction completes, water in collecting tube was removed first, then the essential oil was collected. The collected essential oil was dehydrated by anhydrous sodium sulphate. Then, they were dissolved in 2 ml of acetone to form the reducer as well as stabilizer for the synthesis of AuNPs.

2.2. Synthesis of N. sativa essential oil mediated gold nanoparticles (NsEO-AuNPs)

Chloroauric acid ($HAuCl_4.3H_2O$) (99.99%) was procured from Sigma–Aldrich, Mumbai and used for nanoparticle synthesis. The synthesis of *Ns*EO-AuNPs was carried out following the method of Kumar et al. [8]. About 2 ml of *N. sativa* EO was added to 30 ml of 1 mM aqueous 2.5 $x10^{-4}$ M $HAuCl_4$ solution at 100 °C with continuous stirring and boiled for one minute. Rapid reduction of Au^{3+} ions to Au^0 occurred and the colour of the solution become pink colour. The experiment was repeated with different volumes (5 and 8 ml) of diluted oil to get purple and violet coloured solutions.

2.3. Characterization of NsEO-AuNPs

2.3.1. UV–vis spectroscopy

The bioreduction of the $AuCl_4$ ions in solution was monitored by measuring the UV–vis spectra of the solution in quartz cuvettes with Varian, Cary 50 UV–vis spectrophotometer in the range of 400–800 nm at different time intervals.

2.3.2. X-ray diffraction (XRD) analysis

The particle size and nature of *Ns*EO-AuNPs were determined using XRD (Shimadzu XRD – 6000/6100 models). X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of crystalline material and can provide information on unit cell dimensions. The analyzed material has been finely ground, and the average bulk composition was determined. The grain size of the gold nanoparticles was determined using Debye Sherrer's equation.

$$D = 0.94\lambda/B\cos\theta$$

where λ is the wavelength (Cu Kα), β is the full width half-maximum (FWHM) of the AuNPs (101) line and θ is the diffraction angle.

2.3.3. Fourier transform infrared spectroscopy (FTIR)

Two milligram of the sample was mixed with 200 mg KBr (FTIR grade) and pressed into a pellet. The pellet was placed into the sample holder and spectra were recorded in FTIR spectroscopy (Thermo Scientific Nicolet-iS5) at a resolution of 4 cm^{-1}.

2.3.4. Transmission electron microscopy (TEM)

The nanoparticles were mounted on the copper stubs, and the images were studied using Transmission electron microscope (TEM), HITACHI (Model: S-3400N) with secondary electron detectors at an operating voltage of 20 kV and for elemental analysis.

2.4. Antibacterial activity of NsEO-AuNPs

2.4.1. Collection and culture of bacteria

Gram positive *Staphylococcus aureus* (MTCC 9542) and Gram negative *Vibrio harveyi* (MTCC 7771) were purchased from Microbial Type Culture Collection (MTCC), Chandigarh. Nutrient Broth (NB) and Tryptic Soy Broth (TSB) were used for culturing the bacterial organisms. All strains were stored at −20 °C in the appropriate medium containing 10% glycerol and regenerated twice before use in the manipulations.

2.4.2. Disc diffusion method

The antibacterial activity of *Ns*EO-AuNPs against *S. aureus* and *V. harveyi* was analyzed by disc diffusion method [15]. Bacterial suspensions (10^5 CFU ml^{-1}) were inoculated in Mueller Hinton Broth at 37 °C for 8hr (Himedia, Mumbai). 100 μl of the grown culture were plated on Mueller-Hinton agar using a sterile cotton swab. Different concentrations of *Ns*EO-AuNps (3 μg ml^{-1}, 5 μg ml^{-1} and 10 μg ml^{-1}) loaded in sterile discs were placed on the seeded plates and were incubated at 37 °C for 24 h. After incubation, the zone of inhibition was measured. The experiment was done in triplicate, and the mean value was calculated.

2.4.3. Determination of minimum inhibitory concentration

The minimal inhibitory concentration (MIC) of *Ns*EO-AuNPs was determined using the tube dilution method [16]. Briefly, tubes with 5 ml of Luria–Bertani (LB) broth containing various concentrations of *Ns*EO-AuNPs ranging from 2 μg ml^{-1} to 10 μg ml^{-1} were inoculated with 100 μl of 10^5 CFU mL^{-1} of standardized suspensions of bacterial culture. The tubes were incubated in a shaker (180 rpm) at 37 °C for 24 h. Control tubes without tested samples were assayed simultaneously. About 100 μl from each tube dilution was plated in the MHA plates and incubated at 37 °C overnight and the results were recorded by comparing plates with the control plates.

2.5. Antibiofilm activity of NsEO-AuNPs

The inhibition of biofilm growth of *S. aureus* (MTCC 9542) and *V. harveyi* (MTCC 7771) by *Ns*EO-AuNPs was determined through microtitre plate assay. Bacterial colonies (1×10^6 cfu ml^{-1}) were allowed to grow on glass slides (diameter 1 × 1 cm) placed in 24-well polystyrene plates with 1 ml of nutrient broth supplemented with different concentrations of *Ns*EO-AuNPs (20 μg ml^{-1} to 80 μg ml^{-1}) and incubated for 24 h at 37 °C. Glass slides were

Download English Version:

https://daneshyari.com/en/article/3416369

Download Persian Version:

https://daneshyari.com/article/3416369

Daneshyari.com