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Mechanistic study of antibacterial activity of biologically synthesized silver nanocolloids

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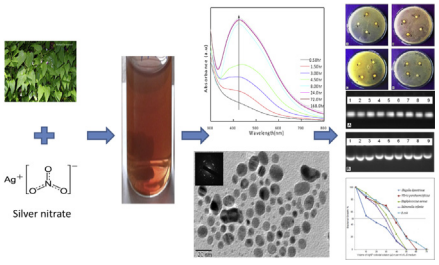
HIGHLIGHTS

- Very stable aqueous silver nanocolloids have been synthesized using single step green biosynthetic method.
- TEM micrograph reveals that the size of the synthesized spherical silver nanoparticle is 8.9 ± 3.6 nm.
- The as-synthesize silver nanocolloids show significant antibacterial activity against several bacterial strains.
- In-vitro and in-vivo DNA damage assay show silver nanocolloids have no genotoxic effect.

GRAPHICAL ABSTRACT



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ABSTRACT

Aqueous silver nanocolloids were synthesized using single step and completely green biosynthetic method employing aqueous leaf extracts of *Paederia foetida* as both the reducing and capping agent. Crystalline silver nanoparticles (AgNPs) having average diameter (8.9 ± 3.6) nm have been obtained. The nanocolloids are very stable and no precipitation was observed in 6 months. The absorption spectra of colloidal silver nanoparticles showed characteristic surface plasmon resonance (SPR) peak centered at a wavelength of 424 nm. The activity of the AgNPs colloidal suspension as an antibacterial agent against both gram negative (*Shigella dysenteriae*, *Salmonella infantis*, *Vibrio parahaemolyticus* and *Escherichia coli*) and gram positive (*Staphylococcus aureus*) bacteria was investigated following MIC as well as disc diffusion technique. Good antibacterial activity was found against all test bacteria. In-vitro and in-vivo DNA damage assay was performed by silver nanocolloid. Interestingly silver nanocolloid showed no genotoxic effect and this results lead to conclude that inhibition of bacterial growth occurred without any DNA damage by silver nanoparticle.

1. Introduction

The antimicrobial properties of metals were known since ancient times [1,2]. Especially silver is traditionally well-known antimicrobial materials and it is less toxic to human cells and environmentally benign in low concentration [3,4]. For this reason silver was used as antiseptic materials specifically for the

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treatment of open wounds and burns before the introduction of antibiotic therapy and silver containers have been used for centuries to purify potable water [5–7]. Along other unique properties such as electronic, optical, catalytic etc metal nanoparticle also exhibit significant antimicrobial activity. The enhanced antimicrobial effect of metal nanoparticle is owing to their high surface to volume ratio, which allows the nanoparticle to interact closely with the microbial membrane [8]. Several studies have been reported on the antimicrobial activity of silver nanoparticle of various size and shape against different antimicrobial strain [9–11]. In most of these studies silver nanoparticles have been synthesized by reduction of silver ions in aqueous phase using environmentally benign reagents such as biological reducing agents and stabilizer [12,13,4]. The advantage of such 'green synthesis' is that the as-synthesized silver nanocolloids can be directly introduced in the application fields as the colloids do not contain any toxic reducing agent and stabilizer. Moreover, the method is also low cost. The use of silver nanoparticles as an antimicrobial agent has been already practiced in several application fields including cloths, fabrics, washing machines, water purification, toothpaste, deodorants, filters, kitchen utensils, toys, wound dressings etc and biomedical areas [14–16]. Very recently some of us successfully fabricated antibacterial and antifungal silver-gelatin nanocomposite as a prospective applicant for bio-packaging [17].

However, very few studies have been reported on the mechanistic pathway of antimicrobial action of silver nanoparticle [18–21]. But such studies are important to know the toxic effects of silver nanoparticle at that particular concentration on human cells.

In the present study, initially we have synthesized very stable silver nanocolloids in aqueous phase by employing plant extract as a reducing and stabilizing agent. The leaf extract of *Paederia foetida* has been used in this purpose. The antibacterial activity of such colloidal silver nanoparticle was tested against several bacterial strains by 'spectroscopic analyses and 'zone of inhibition' techniques. Minimum inhibition concentration (MIC) is also reported for each bacterial strain. To know the killing-mechanism, we have investigated the effect of silver nanocolloid on calf thymas (CT) DNA (in-vitro) and on genomic DNA of *Escherichia coli* (in-vivo).

2. Materials and methods

2.1. Preparation of leaf extract

P. foetida was collected from Sonarpur, Kolkata-700150, West Bengal. The fresh leaf extract was prepared by taking 50 g of thoroughly washed and finely cut leaves in a 500 ml flask with 100 ml of sterile distilled water and then boiling the mixture for 5 min before finally filtering it through Whatman No. 1 filter paper. The extract was stored at 4 °C and used within a week.

2.2. Synthesis of silver nanocolloids

Silver nitrate ($AgNO_3$, 99.99%) was purchased from Sigma. For a typical synthesis, 0.40 ml of leaf extract was added to a 5 ml of freshly prepared 1 mM aqueous $AgNO_3$ solution. The mixture was taken in a sealed Teflon container and it was kept in an incubator with vigorous shaking at 50 °C for maximum 7 days. The volume of leaf extract was varied from 0.16 to 0.48 ml where the concentration and amount of $AgNO_3$ solution was kept constant. The final volume of the solution mixture was maintained 6 ml in each set.

2.3. Characterization of AgNPs

The UV–vis absorption spectra were measured as a function of reaction time at room temperature on a Perkin Elmer UV spectrophotometer (model λ-25) using a quartz cell (1 cm path). 24 h aged sample was taken for TEM and antimicrobial study. The TEM study was carried out in a JEOLJEM 2100 microscope, working at an acceleration voltage of 200 kV. Sample for TEM analysis was prepared by placing a small drop of colloidal solution on carbon coated copper grid. After 2 min of deposition of the film on TEM grid, the excess solution was removed using a blotting paper and the grid was allowed to dry in room temperature prior to measurement.

2.4. Microbial strains used and growth conditions

The microorganisms used in this study were clinical isolates of *S. dysenteriae*, *V. parahaemolyticus*, *S. aureus*, *S. infantis* and *E. coli*.

Bacterial strains stock cultures were maintained at 4 °C on nutrient agar medium. Active cultures were prepared by inoculating fresh nutrient broth medium with a loopful of cells from the stock cultures at 37 °C for overnight. To get desirable cell counts for bioassays, overnight grown bacterial cells were sub-cultured in fresh nutrient broth at 37 °C. In vitro antimicrobial activity of the silver nanocolloid was screened against a total of five above mentioned bacterial strains.

2.4.1. Determination of MIC by spectroscopic analysis

The silver nanocolloids were later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial strain. Freshly, grown bacterial strains 100 μl (1×10^6 cells/ml) in nutrient broth was inoculated in tubes with nutrient broth supplemented with different concentrations (0–100 μl/ml) from the stock sample (0.899 μg/ml) and incubated for 24 h at 37 °C. Presence of turbidity denoted presence of micro organism in the test tube after the period of incubation whereas the complete absence of any turbidity indicates complete inhibition of microbial growth. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC. The MIC was calculated for the individual bacterial species.

2.4.2. Disc diffusion method

The antimicrobial activity of silver nanocolloids was screened using disc diffusion technique [17]. The agar plates were prepared by pouring 15 ml of molten nutrient agar media into sterile petriplates. The plates were allowed to solidify and 0.1% inoculums suspension was swabbed uniformly with sterile cotton and was allowed to stand for 15 min. The different dilutions of the test sample (0%, 25%, 50% and 75%) from stock concentration (89.9 μg/ml) were loaded on 6 mm autoclaved filter paper discs. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were incubated at 37 °C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with ruler in millimeter. These studies were performed in triplicate.

2.4.3. Determination of genotoxic effect of silver nanoparticles on the in-vitro damage of genomic DNA

50 μg of Calf Thymus (CT) DNA and varying concentration (0–100 μl/ml) of silver nanocolloids was mixed, and incubated at 37 °C for 1 h. After treatment, the DNA was immediately subjected to electrophoresis in 0.9% agarose gel, in 1× TAE buffer (40 mM-Trise-acatate, 1 mM EDTA), followed by ethidium bromide staining (50 μg/ml) and visualized by gel documentation system. The resulting fragmentation and inhibition of fragmentation were analyzed.

2.4.4. Determination of in-vivo genotoxic effect of silver nanocolloids on E. coli cells

1×10^6 cells/ml of *E. coli* were treated with and without silver nanocolloids (10–100 μl/ml) and incubated at 37 °C for 24 h. After the incubation is over, cells were treated with 200 μl cell