In Vitro and In Vivo Effects of Silver Nanoparticles Biosynthesized By Serratia Marcescens

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Abstract

Biosynthesis of silver nanoparticles by Serratia marcescens was demonstrated using the supernatant for extracellular biosynthesis. The formation of AgNPs was characterized by UV-Vis spectrophotometry, atomic force microscopic (AFM) and fourier transform infrared spectroscopy (FTIR). Antimicrobial activity of silver nanoparticles was performed by use well diffusion method and show that these AgNPs can be used as growth inhibitors against pathogenic isolates: Salmonella sp., Staphylococcus aureus, Staphylococcus epidermis, E. coli, Klebsiella sp, Candida albicans . Antibiotic susceptibility of Salmonella sp. was determed by used disk diffusion method and found the most susceptible for amoxicillin, chloramphenicol, azithromycin, ampicillin and cefotaxime and resistance to flumequine neomycin and colistin. The present study was designed to assess the effect of silver nanoparticles wich synthesized by serratia marcescens in modulating the salmonellosis in BALB/C mice. we found that in vivo the histological study for liver it was damage when compared with mice’s liver treated with antibiotic (amoxicillin).

Keywords: Serratia marcescens, silver nanoparticles, pathogenic bacteria, liver.

Introduction

A nanoparticle is a microscopic particle with at least one dimension less than 100 nm. Usually ranges from( 1-100) nm for application of nanoscale materials and structures. Nanoparticles are of great scientific interest as they are effectively a bridge between atomic or molecular structures and bulk material [1].

They are widely used as an additive for numerous products and materials including ceramics, plastics, cement, glass, rubber, pigments, ointments, lubricants ect.[2].

Chemical and physical methods for nanoparticles synthesis are expensive and involve the production of toxic products which are not safe for environment. Many microorganisms such as bacteria and fungi have been used in synthesis of metallic nanoparticles [3].

Nanoparticles synthesized using microbes are also called “green synthesis”. Various microbes can reduce the Ag+ ion to form silver nanoparticles [4]. Silver nanoparticle has unrivaled properties which help in devices that are used in several medical procedures, as well as in molecular diagnostics and in therapies. Screening for potential toxicity of incorporation nanoparticles into consumer products is necessary to ensure customer safety liver is an important organ for accumulation. Ag NPs are highly relevant for human exposure due to their use in food contact materials, antibacterial wound treatments and dietary supplements [5].

For the synthesis of metal nanoparticles, many studies were focused on extracellular methods [6]. Serratia marcescens is a rod shape, Gram negative bacteria in the family Enterobacteriaceae [7].

In the present study , a green approach to synthesized nanoparticles using bacterial cell filtrate of locally isolate of serratia marcescens and antibacterial activity for this nanoparticles against some pathogenic bacteria in vitro and study histopathological effects for silver nanoparticles on liver mice and compared with histopathological effects of antibiotic.

Materials and Methods

Microorganism

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Salmonella sp. Staphylococcus aureus, Staphylococcus epidermedis, E.coli, Klebsiella sp., Candida albicans. And serratia marcescens isolates were obtained from AL-Kindiy Hospital in Baghdad/ Iraq. These isolates were identified by Conventional biochemical reaction according to the criteria established by Forbes et al. [8].

**Antimicrobial Susceptibility Tests**

The disk diffusion method was performed to determine susceptibility of the *salmonella sp.* isolate based on the NCCLS 1996 protocol (National Committee for Clinical Laboratory Standards).

The bacterial suspension turbidity was adjusted to McFarland standard number 0.5 in Mueller Hinton broth and cultured fluently over the entire surface of Muller Hinton agar with sterile cotton swab. Commercial antibiotic disks containing single concentrations of each antibiotic were then placed onto the inoculated plate surface. The zone of inhibition of growth around each disk after overnight incubation at 37 °C was measured in millimeters. The zone diameter was interpreted using a zone size interpretation chart [9].

The antimicrobial agents tested and the corresponding concentrations were as follows: amoxicillin 30 μg, chloramphenicol 30 μg, cefotaxime 03 μg, flumequine 50μg, colistin 10 μg, neomycin 30 μg, azithromycin 15 μg, and ampicillin 30 μg.

**Biosynthesis of Silver Nanoparticles by Serratia Marcescens**

Nutrient broth was prepared, sterilized and inoculated with a fresh growth of test strain *serratia marcescens*. The culture flasks were incubated at 30°C for 24h in an orbital shaker at 150 rpm. After the incubation period, the culture was centrifuged at 10,000 rpm for 5 min and the supernatant was used for the synthesis of silver nanoparticles (Ag NPs). The supernatant of *serratia marcescens* culture was mixed with 1 MM silver nitrate solution and incubated at 35 C° for 72h [10].

**Characterization of Biosynthesized Silver Nanoparticles**

The biosynthesis of silver nanoparticles which produced by locally isolate *serratia marcescens* characterized by using UV-Vis spectrophotometer (PC Based Double Beam Spectrophotometer, 2202, Systronic), Atomic Force Microscopic (image was taken using Park system AFM XE 100) and was confirmed the presence of elemental silver and Fourier transforms infrared spectroscopy (FTIR, BRUKER Vector 22 Spectrophotometer) was analyzed the interaction between protein–silver nanoparticles.

**Antimicrobial Activity of Ag NPs**

Antimicrobial activity of biosynthesized Ag NPs by serratia marcescens isolate were tested against *salmonella sp.* Staphylococcus epidermedis, E.coli , Klebsiella sp., Candida albicans. Isolates by used well diffusion method [12].

To determine the antibacterial activity, a small volume of sterile water was poured inside a test tube to which general colonies of the test bacteria taken directly from plate were emulsified by adding sterile distilled water, the suspension was adjusted to match 0.5 McFarland’s Standard.

A half ml volume of the suspension was spread over the plates containing Muller–Hinton agar using sterile cotton swab in order to get a uniform microbial growth. Well of 6 mm diameter were made on Muller –Hinton agar plate using sterile cork – borer, 50μL of AgNPs solution (5,10 mg/ml), supernatant without AgNO3, and ofloxacin (2mg /ml ) as a control separately was poured onto each well .The plates were incubated at 37°C for 24hrs; the diameter of zone of inhibition was measured in millimeter.

**Experimental Design**

Twenty –four male albino mice aged 12-13 weeks weighing 15-17 gm were obtained from the animal house in collage of medicine Baghdad university were housed under standard condition then were divided mainly into four groups of six animals each as follows:-

Group 1 (G1): (positive control group)-were fed orally with a single dose of respective *Salmonella sp.* (0.1 ml) contain 1×10⁹ CFU. [13]. for 7 days.

Group 2 (G2): (Treatment with amoxicillin)-these mice were challenged orally with a single dose of *Salmonella sp.* (0.1 ml) contain 1×10⁹ CFU, after3 day, mice fed orally with a
sing single dose of amoxicillin 0.1 ml (0.25mg/ml) for (10) days.

Group 3 (G3): (Treatment with silver nanoparticles) - as well, fed single dose of salmonella spp., after 3 days give orally with a single dose of silver nanoparticles 0.1ml (5 mg / ml) for (10) days

Group 4 (G4): (control) - also fed with a same dos of silver nanoparticles . A 3days after fed with a single dose of PBS-7.2 via orogastric gavages along the period of experiment.

For LD50 calculations, different of concentration of silver nanoparticles (5, 10, 15 mg/ml) were inoculated (0.1 ml) through a gastric tube (n 6 mice/inoculum). The health of the animals was monitored for 30 day after inoculation, and deaths were recorded. [14].

Histopathological Study

Mice were dissected and liver was removed and fixed with formalin embedded and section stained with hematoxylin-eosin. [15]. were then examined by light microscopy.

Result and Discussion

Antimicrobial Susceptibility Tests

Salmonella sp. Isolate in vitro was susceptible to chloramphenicol, azithromycin, cefotaxime, amoxicillin and ampicillin, while resistance to flurmequine, colistin, and neomycin. According to this result we choose amoxicillin to treat salmonellosis in vivo.

Biosynthesis of Silver Nanoparticles by Serratia Marcescens

Serratia marcescens isolates were tested for synthesis of silver nanoparticles. Extracellular biosynthesis of silver nanoparticles by the supernatant when add 1Mm AgNO3. Visual watching showed a change of colour in supernatant from yellow to brown (fig.1), while no colour change was noted in the culture supernatant without silver nitrate or in media with silver nitrate alone. The release of brown colour in silver nitrate treated culture supernatant suggested the fashioning of silver nanoparticles [16].

A similar observation was made by [17] . In the biosynthesis of Ag –NPs by serratia marcescens strain by extracellular process. The excitement of surface Plasmon vibration of Ag –NPs, the change colour of the medium to brown could be consequent [18] . The mechanism of biosynthesis of Ag-NPs is exacting known, however, it has been assumption that silver ions desired the NADPH-dependent nitrate reductase enzyme for their reduction which was produced by the bacteria in its extracellular environment [19].

Characterization of Synthesized Silver Nanoparticles

UV- Vis Spectral Analysis

The UV-Visible spectrum of supernatant reaction with 1mMAgNO3 solution recorded was plotted as shown in Figure (2) ,it was showed an surface plasmon resonance ( SPR) peak of nanoparticles at (420) nm . this observation indicates the protein was released into the supernatant and suggests a possible mechanism for the reduction of the metal ions present in the solution [20].
Fig:2: UV.Vis spectrum of AgNPs

Atomic Force Microscopy

By atomic force microscopy in this study converted particle size was analysed. AFM was used to show the nanoparticle both in surface and three dimensional views (Fig. 3), and found the average size of particles 93.55 nm. The image gave the clear shape and size of the AgNPs synthesized by serratia marcescens, while Lakshmipathy and Nanda,[ 17] found the particles size ranges from 30-70nm. Metal nanoparticles a wide size range of 2-100 nm for some genus of Enterobacteraeceae [21]

Fig: 3: Atomic Force Microscopy image of silver nanoparticles synthesized by serratia marcescens
FTIR Analysis

Fourier transform infrared spectroscopy (FTIR) was using to identify the functional group of protein molecule surrounding the silver nanoparticles.

The FTIR spectrum of biosynthesized silver nanoparticles by *serratia marcescens* shown in figure (4) the strong peak observed at 3853.46 (400-4000), as similar with the result of [22] the peak at 3750.13 and 3853.46 refers to O-H, the 2959.58 refers to C-H stretching vibration. The peak seen at 2356.04 are identified as the amine group. The peak at 1634.37 refers to C-O stretching vibration mode. The peak 1455.55 refers to amino methyl stretch. The peak 1080.19 refers to ether linkage. The peptides and the carbonyl groups of the amino acid residues have strong ability to bind to the silver [23]. It also reported that the free amine or cysteine groups in proteins can bind to nanoparticles, and may acts as capping agent for stabilization of nanoparticles [24].

**Figure 4: FTIR spectrum of silver nanoparticles biosynthesized by *Serratia marcescens* **

Antimicrobial Activity of Silver Nanoparticles

Antimicrobial activity for the silver nanoparticles biosynthesized by *serratia marcescens* were tested against *Salmonella sp.*, *Staphylococcus aureus*, *Staphylococcus epidermedis*, *E. coli*, *Klebsiella sp.*, *Candida albicans*, using well diffusion technique. The diameter of inhibition zones around each well with different concentration of silver nanoparticles (5, 10 mg/ml) and control antibiotic Ofloxacin is present in (Table 1). The well with high concentration it was with high zone inhibition (fig 5) this result suggest that silver nanoparticles synthesized by *serratia marcescens* could be used as an effective antimicrobial material in medical field. The surface modified nanoparticle with a positive charge has a greater affinity toward the negative charge of bacterial cells [25]. The antimicrobial action of silver nanoparticles is linked with four well-defined mechanisms: 1-adhesion to cell wall of microbe 2-penetration and damaging intracellular structures 3-induced cellular toxicity and 4-AgNPs modulation of signal transduction pathways [26]. Many research reported that silver nanoparticles have antimicrobial effects against pathogenic bacteria and fungi [27].

**Table 1: Antimicrobial activity of AgNPs**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition (mm)</th>
<th>AgNPs</th>
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<tr>
<td></td>
<td></td>
<td>5 mg/ml</td>
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<tr>
<td>Control</td>
<td>Supernatant without</td>
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<tr>
<td>Bacteria</td>
<td>(ofloxacin) 2mg/ml</td>
<td>AgNO3</td>
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<tr>
<td>Staphylococcus aureus</td>
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<td>Staphylococcus epidermidis</td>
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<tr>
<td>Salmonella sp.</td>
<td>16</td>
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<tr>
<td>E. coli</td>
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<tr>
<td>Klebsiella sp.</td>
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<td>Candida albicans</td>
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- (ofloxacin) 2mg/ml
- AgNO3
Histopathological Study

Study the effects of silver nanoparticles and antibiotic (amoxicillin) on liver tissue of mice was studied and carried out, after 21 days removed the tissue from the mice and staining by hematoxylin and eosin then compared with the control group.

The results show that silver nanoparticles causes proliferative of kupffer cells, hemorrhage and infiltration of lymphocytes cells (Fig.8) compared with negative controle group shows normal tissue (Fig.6) and with (amoxicillin) group shows microgranulomas are formed in the liver parenchyma, sinusoidal sinusis and necrosis and hemorrhage (Fig .7).

The liver is one of the most important targets after exposure to AgNPs, as major organ of detoxification. In vivo, we found the silver nanoparticles it was damage the liver when compared with mice liver treated with antibiotic. In vivo, Gaiser et al. [14] We found smaller agglomerates particles in the cytoplasm, which were not visible under the light microscope The presence of Ag NPs in the nucleus suggests that at least some of the particles were initially free within the cytoplasm and did not appear to be membrane bound and we Found the silver nanoparticles are lethal to zebrafish so, Monack et al. [28] Found the main toxicological concern is the fact that Ag NPs preferentially accumulate in mitochondria.
Figure (7): Liver tissue of mice treated with antibiotic causes Microgranulomas are formed in the liver parenchyma ←, hemorrhage ↓ and sinusoidal sinuses →. H&E.40X

Figure 8: Liver tissue of mice treated with nanoparticles causes infiltration of lymphocyte ←, hemorrhage ↓ and proliferative of kupffercells →. H&E.40X

Conclusion
This study demonstrated extracellular biosynthesized of silver nanoparticles by *serratia marcescens*. the biosynthesized Ag NPs have antimicrobial activity against pathogenic microorganism and have side effects on liver compared with antibiotic.

References


