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Antibacterial and Cytotoxic Effects of Silver Nanoparticles on *Staphylococcus aureus* and Normal Vero Cells

Rasha Hadi Saleh, [1] Entisar J. Al-Mukhtar[2*], Zaytoon A. Al-Khafaji[3], Mohammed H. Al Hasnawy[4] , Huda H. Al-Hasnawy[5]

1. College of Pharmacy, University of Babylon, Hilla, IRAQ
2. Dept. of Pharmacology, College of Medicine , University of Babylon, Hilla, Iraq
3. Dept. of Microbiology, College of Medicine, University of Babylon, Hilla, IRAQ.
4. College of Veterinary Medicine, Al-Qasim Green University, Hilla, IRAQ.
5. Dept. of Microbiology, College of Medicine, University of Babylon, IRAQ hudashmm@gmail.com

* **Corresponding author:**

Dr. **Entisar J. Al-Mukhtar**

Dept. of Pharmacology/ College of Medicine

University of Babylon, Hilla, Iraq.

E-mail: ejhas28@yahoo.com

Abstract

Silver nanoparticles (AgNPs) are of special concern as a result of their unique chemical, physical and biological characteristics. It has become an attractive alternative to antibiotics due to their broad-spectrum antimicrobial activity. The study aimed to determine the antibacterial activity of AgNPs against *S. aureus* bacteria and the effect of AgNPs on the viability of normal cell line (vero cell). A total of 70 clinical samples (wound and vagina swab, stool and urine) were used in this study. Bacterial isolates were subjected to the microscopical, cultural and biochemical evaluation. AgNPs were prepared and checked for their antimicrobial activity by the use of various concentrations employing agar dilution method. In addition, the effect of different concentrations of AgNps on a viability of vero cells

was examined. The results showed that out of 70 clinical samples, 11 (15.7%) isolates were *Staphylococcus aureus*. AgNps showed high activity against *S. aureus* **at concentrations (100 µg/ml and 200µg/ml). It was found that there was** no effect of AgNPs **on the viability of the normal vero cells at (≤ 250 µg/ml) concentration, but** they have cytotoxic effect on the viability of the these cells at high concentrations. This study concluded that AgNPs possess good antimicrobial activity and the concentrations that maintain the cell viability could be used as an alternative therapy to treat *S. aureus* infections.

Keywords: nanoparticles, *S. aureus*, cytotoxicity.

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1. Introduction

The increasing number of multiple antibiotic-resistant microorganisms and failure to treat infectious diseases are the main problem in the medical field [1], therefore, many scientists do research to produce new efficient agents that exceed the resistance of these microorganisms and are also cost-effective [2]. Metal nanoparticles showed clear activity against

microorganisms. They possess dimensions of (100nm) or less. The most important property of nanoparticles is their large surface-area to volume ratio [3].Among metal nanoparticles; AgNPs have drawn attention to the scientific area. Silver is traditionally used nanomaterial in shopper products [4]. The most important application of AgNPs in pharmaceutical manufacturing was ointments to interrupt open wounds and burn infection [5].

It has been found that AgNPs are non-noxious to humans and are effective against bacteria at low concentration, thus they don't having any side impacts to human [6].Many studies proposed that AgNPs link to the cell membrane surfaces dispersion permeability and respiration behaviour of the cell [7,8]. *Staphylococcus aureus* is an important pathogen that causes serious complications ranging from petty to life-threatening infections [9].These bacteria represent a leading cause of infections correlated with indwelling medical tools like catheters and artificial heart valves due to ability of *S. aureus* to make biofilms on such materials thus causing persistent infections. Another trouble is the capacity of these bacteria to develop resistance to a several antibiotic therapy[10]. This, in turn, has led to the use of new antimicrobials like AgNPs as an alternative to antibiotics [11]. Therefore, we aimed at this study to test the antibacterial activity of AgNPs against *S.aureus* isolates and assessed the effect of AgNPs on the viability of normal vero cells.

2. Material and methods

2.1. Isolation and identification of *S. aureus* isolates

A total of 70 clinical samples (wound, vaginal and swab, urine and stool) were collected from patients attended Al-Hilla Teaching Hospital during a period of six months. The samples were inoculated on nutrient agar and blood agar, and then incubated at 37°C for overnight below aerobic circumstances. All *S. aureus* isolates were identified according to their diagnostic distinctive and compared with their being recorded in referential references [12,13].

2.2. Preparation of silver nanoparticles(AgNps)

Silver nanoparticleswith (99%) purity were purchased and prepared according to the instructions in source supplied by Hongwu International-China. Serial dilutions of AgNPs were made by two fold dilutions to obtain numerous concentrations. Solutions were sterilized with (0.45mm) millipore filters.

2.3. Antibacterial effect of AgNPs:

The used nanoparticles were of size (20 nm). Based on CLSI, [14], recommendations, the MIC was determined versus *S. aureus* via using the agar dilution method. The bacterial isolates were subjected to sequential twofold dilutions of **AgNPs concentrations from 12.5 to 200µg/ml** and the viability was determined following incubation at 37°C for 24 hours [15].

2.4. *In vivo* cytotoxicity assay (cell culture):

Vero cell line was provided from National Cell Bank of Iran-Pasteur Institute. Cell line was kept in Roswell Park Memorial Institute medium 1640 (RPMI-1640, Gibco-BRL), supplemented with 100 µl/ml penicillin/streptomycin (Sigma). Cells were incubated in 5% CO_2 incubator for 24 hr at 37°C or until a confluent monolayer formed. The cells were subcultured for several times and then seeded into 96-well microplates at concentration of 4 × 105 cells per well with complete RPMI-1640 growth medium.

Exposure stage

Vero cell line was cultured in 96-well-flat bottom microtitration plates and incubated with serial titer dilutions of AgNPs ranging from (31.25-1000) **µg/ml**. The plated cells were split into two groups: Group-1 was the control (untreated) group; group-2 cell line was treated with AgNPs. Three replicate were used for each AgNPs titer dilution, as well as the control group which was treated with serum free medium only. The plates were incubated in 5% CO_2 incubator for 48hrs at 37°C, and then cytotoxicity of AgNPs was estimated.

2.5. Assessment of AgNPs cytotoxicity by crystal violet assay

Crystal violet (CV) assay was used to determine the optical density (OD) of the cell growth in each well of the microtiter plate by using ELISA reader. It is a technique used in cell culture laboratories to detect remaining adherent cells that staining with crystal violet dye, which binds to proteins and DNA. This technique was processed according to method recommended by[16]. The viability of the cell was calculated according to the equation:

GI = OD control – OD test * 100 / OD control

Viability of cell = 100 – GI

3. Results

3. 1. Isolation and identification of *S. aureus* Isolates

Morphological and biochemical characterization of bacterial cultures revealed that among 70 clinical samples, 11(15.7%) were found to be *S. aureus*. The distribution of *S. aureus* isolates according to the site of infection is presented in the (Table11).

Table (1): The Number and percentage of *S. aureus* isolated from clinical specimens

Sources of isolates	**No. of samples**	**No. (%) of *S.aureus* isolates**
Wound	25	5(20%)
Urine	20	1 (5%)
Vagina	15	3(20%)
Stool	10	2(20%)
Total	70	11(15.7%)

3.2. Antimicrobial activity of Silver Nanoparticles (AgNps)

The antimicrobial activity of **various concentrations of AgNps (from 12.5µg/ml to 200 µg/ml) was estimated against** *S. aureus* by agar dilution method. The antibacterial activity was seen at **concentrations 100 µg/ml** and 200 **µg/ml only.**

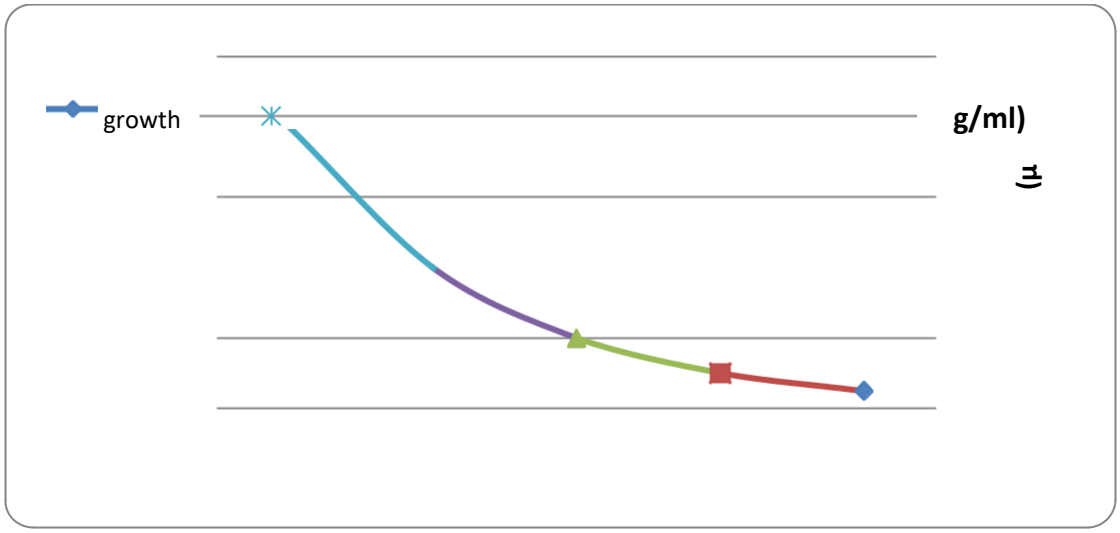

Figure (1): Antibacterial Activity of Various Concentrations of AgNps on *S. aureus*

3.3. The Cytotoxic effect of AgNPs on Cell Viability

To evaluates the safety of (AgNPs) candidates for use in medical field, the cytotoxicity of AgNPs was determined with viability cell assays. To our acknowledgment, the present study was the first in Iraq to determine the cytotoxicity of nanosilver solution on normal vero cell. The present findings showed that nanosilver does not inhibit viability of the normal vero cell at low concentrations(31.25, 62.5, 125, 250) **µg/** ml, but it has toxic effects on the normal vero cell at higher concentr**ations (500, 1000) µg/ml as AgNPs exhibited complete loss of viability at these concentrations (Figure**-1).

4. Discussion

4.1. Isolation and identification of *S. aureus* Isolates

S. aureus is an important cause of community and hospital acquired infections which can lead to various consequences [17].The finding of current study was comparable with that reported by [18] who found that isolation rate of *S. aureus* was (16.66%) with distribution rate as (15.7%) from wound while it was (20.6%) from urine. Also, Saleh, [19] in her study found that *S. aureus* detected in 6(21.4%) of wound samples. Furthermore, Dilnessa and Bitew [20] stated that the rate of isolation of *S. aureus* isolates from vaginal discharge, urine and stool was (2.5%), (2.8%) and (1.8%) respectively. *Staphylococcus aureus* is responsible for serious infections like skin infections (furuncles, impetigo, boils), burn and wounds infections, intestinal and urinary tracts infections, osteomyelitis, meningitis, endocarditis, toxic shock syndrome, and septicemia [21]. It's capable of prolonged survival on a variety of environmental surfaces, it can be survive in distilled water and in all parts of hospital moreover it is resistant to chemical disinfectants and many of conventional antibiotics [22].

4.2. Antimicrobial activity of Silver Nanoparticles (AgNps)

Silver nanoparticles have powerful antimicrobial properties and it is recorded to have an antimicrobial effect against *S. aureus* **at concentrations (100 µg/ml and 200µg/ml).** These results were in accordance with several authors worldwide [23-25] who stated tha AgNPs exhibited antimicrobial1activity against *S. aureus* **at a concentration (100 µg/ml), While**
[7] stated that *S .aureus* growth **was inhibited at higher concentration reached 800 µg/ml AgNPs. The bacterial growth**

inhibition by AgNPs was found to be concentration-dependent as the antibacterial activity was elevated with the increase- of (AgNPs) concentration. Many authors worldwide (2,10, 26,27) revealed that AgNPs possess a potent antimicrobial activity against *S. aureus* bacteria.

The AgNPs have bactericidal effect against a wide spectrum of pathogenic bacteria (28). AgNPs exert efficient growth inhibitory effect as a result to their large surface area which providing enough connection with microorganisms[3]. The hypothesized mechanisms of antibacterial activity of AgNPs, mainly related to the disruption of the bacterial cell membrane and exhaustion of intracellular level of ATP and the outcome is suppressing of respiratory enzymes[10]. Infectious bacteria are improbable to promote resistance toward silver because this metal has a negative impact on a wide range of targets in the bacteria, suggesting that pathogens have to evolve a high number of mutations concurrently to protect themselves [29].

4.3. The Cytotoxic effect of AgNPs on Cell Viability

The present study demonstrated that the viability of cells decreased at high concentrations of nanosilver.Ali *et al*., [30] stated that Nano-Ag exhibited lower cytotoxicity toward normal cells (M-Stem cell and human fibroblasts (HF2). Also, Kawata, *et al*. [31] study which evaluated the toxicity of AgNPs on human hepatocytes found that nanosilver does not has toxicity at low concentrations, but it has toxic effects on human hepatocytes at high concentrations. Also another study Taleghani *et al*. [32]found that viability of human gingival epithelial cells was significantly decreased at high concentrations of AgNPs under *in-vitro* conditions. Different toxic concentrations reported by previous studies may be due to different preparation method of silver nanoparticles or size of the particles. A direct prediction and comparison in activity (on bacteria) and toxicity (versus human cell lines) is possible [33]. The dose of AgNPs which inhibits inhibit the bacterial growth without causing an injury to **the host cells was (100 µg/ml) [**26].Results suggested that Nano-Ag has antimicrobial activity against *S. aureus*, with low toxicity to the body, thus they can be effectively used in the development of silver-based creams and ointments for curing staphylococcal lesions, new types of disinfecting solutions, silver-containing dressings in treating burns, dressing endotracheal tubes and urinary catheters [29].

Conflict of interest

None of the authors have any conflicts of interest relevant to this research subject.

Ethical Approval

Ethical Committee of the Babylon health directorate approved the study. All patients' consents were taken before inclusion in the study.

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