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The effect of Silver nanoparticles in the treatment of *Pseudomonas aeruginosa* infections

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Abstract

This study proves that silver nanoparticles can be developed as an antimicrobial agent against *Pseudomonas aeruginosa*, using three strains of microorganisms in this study. Antimicrobial effect was studied using disk diffusion technique. An inhibition area (4.8 cm) was observed with a dose (10 mg) of nanoparticles compared with gentamicin (250 mg) and was inhibition zone (2.2 cm).

Keywords: Silver Nanoparticles, *Pseudomonas aeurginosa*, Antimicrobial agent

INTRODUCTION

Pseudomonas aeruginosa Gram negative bacteria, it is considered a serious pathogen for human health, one of the most opportunistic pathogens that cause high morbidity and mortality (1).

Supports bacterial communities in an extracellular matrix linked to a surface. The microbial number that contains biofilm can consist of single or multiple bacterial species. Bacteria show biomembranes on different surfaces such as natural water systems - living tissue - and other organs, (2), (3).

An extracellular matrix is an intermediate mass of bacteria that focuses on a three-dimensional biofilm structure and mediates bacterial support, (4).

The composition of the matrix - which directly affects the structure of the biofilms - is controlled by enzymes secreted in response to the nutrient nest, (5).

The bacterial adhesion to the surface becomes irreversible, and this is consistent with changes in physiology - gene expression and protein appearance in cells.

In the cells of the surrounding area are used, structures are connected to objects and become an integral part of the extracellular matrix. In the early stage of the formation of the biofilms, the attached bacteria are duplicated and distinct micro cells are used from the surrounding area, linked to the structures of objects and become an integral part of the extracellular matrix. At an early stage of biofilm formation, the attached bacteria are replicated and the composition of the microcolonies is distinct. The steps leading to the development of biofilms were identified in the design, (6).

Nanomaterials for the formation of biofilms depend on the use of nanoparticles, which cover the surface of biomaterials (7), impregnation (8) or the inclusion of nanomaterials (9). One of the most powerful technologies is the use of nanochemistry for nanotubes that are more active and effective than conventional antibiotics against drug-resistant pathogens. The reason for the increased affinity of these nanoparticles may be due to the permeability of the label through the cell cover, (3).

The objective of our study is to investigate the best ability of silver nanoparticles on infection therapy by *Pseudomonas aeruginosa*.

MATERIALS & METHODS

Materials:-

i. Bacterial strains: Three strains of pseudomonas aeruginosa were taken from the Faculty of Science / Biology Department / University of Mosul. They were diagnosed using biochemical tests. The strains were inhibited in the brain-heart injection broth and incubated at 37 ° C under 24-hour air conditions. Then stored at 4 ° C in the refrigerator until use, (10) .

ii. Preparation of silver nanoparticles: These particles were obtained from Sigma-AL-DRICH CO (Germany) (powder) and prepared on (11) and (12).

iii. Laboratory animals: - We used 9 mice in this study, ranging between 1.5-2 months and their weight ranged from 50 g.

Methods:-

Our study consisted of:-

A. In vivo experimental study.

B. In vitro experimental study.

In vivo experimental study included:-

The rats were infected with an inoculum of *Pseudomonas aeuginosa* at concentration of cfu /ml. Intraperitoneally, and these rats were left for the following 3 days to ensure the occurrence of infection.

The (9) infected rats were divided into 3 groups (3 rats / group).

- The Group A was fed with Silver Nanoparticles (10 mg) by mixing these particles with ration daily for (5) days.
- The group B treated with Gentamicin (250 mg) in drinking water twice daily for (5) days.
- The group C was served as the control without inoculated with *Pseudomonas aeruginosa* and was fed with Silver nanoparticles only.

B. In vitro experimental study included :-

1- *Pseudomonas aeruginosa* was inoculated in brain heart infusion broth, incubated for 24hrs. At 37 ^{o}c.

2- Muller- Hinton agar plates were seeded with (0.1) ml of liquid inoculum prepared for the strains of *Pseudomonas aeruginosa*. Three discs were prepared, one impregnated with Silver Nanoparticles (10 mg), the second with Gentamicin (250 mg) and third one with saline as control, these discs were put on the plates, incubated at 37^{o}C for 24hrs. Results were recorded after incubation.

RESULTS & DISCUSSION

The results of a pilot study in vivo showed that silver nanoparticles had antibacterial activity against *Pseudomonas aeuginosa*.

Significant differences in infection rate were observed between infection treated with saline solution and infections treated with either nanoparticles or antibiotics, and silver nanoparticles gave the highest recovery rate compared to other groups. The same results were obtained from the experimental study in the laboratory, the inhibition zone against *Pseudomonas aeruginosa* was greater with the particles than with the antibiotic. - Control disk (saline) showed a non-inhibitory effect image (1) and table (2). Also the results reported in the figure (3) indicate that the first group treated with Silver nanoparticles recovered after 120hrs. , while the second group treated with Gentamicin recovered after 72 hr.

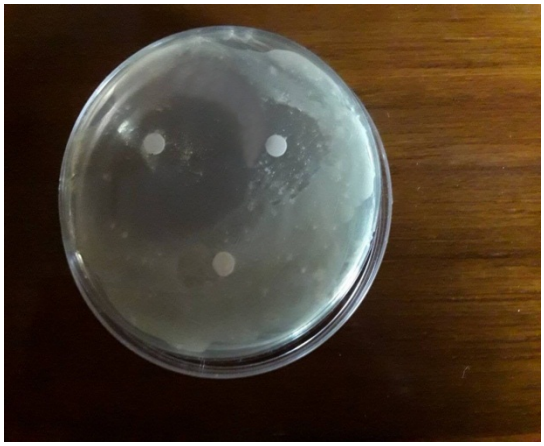

Figure (1) Showed zone of inhibition of
A= Silver Nanoparticles . B= Gentamicin (250 mg).C= Saline against .*P seudomonas aeruginosa*

Table (2) Showed zone of inhibition of A= Silver nanoparticles . B =Gentamicin (250 mg) .C= Saline against .*Pseudomonas aeruginosa*

Treatment	Zone of inhibition (cm)		
Silver (A)	4.8		
Gentamicin(B)		2.2	
Saline(C)			0

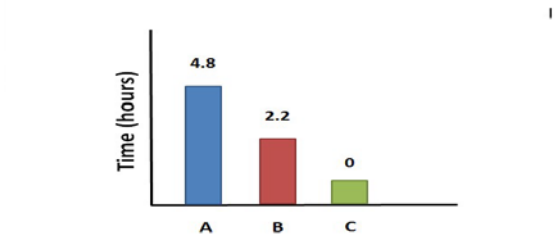

Figure (2) Relation between time of recovery and type of treatments.
S= Silver nanoparticles A= Gentamicin (250mg) C= Control (Saline)

There are studies on the mechanism of action of silver nanoparticles that silver ions penetrate bacterial cells, fibrillation ribosomes and secrete the expression of the enzymes and proteins necessary for the production of ATP, which leads to cell disruption. 13 - The study also showed that nanoparticles have an antibacterial effect on *Pseudomonas aeruginosa.*

The data presented in this work is good and therefore can lead to new progress in the development of antibacterial substances that can be overcome. However, further toxicity studies and potential negative effects that can arise in the use of silver nanoparticles as an effective antibacterial agent in health should be undertaken (14).

The microbial activity of nanoparticle particles against multidrug-resistant bacteria can lead to a range of applications of these nanoparticles in the maintenance of food, disinfection of medical supplies and equipment, and silver nanoparticles can be thought of as effective effective agents of widespread bacteria

Another recent study, silver nanoparticles appears to have been well-designed in the removal of pseudomonas aeruginosa, it has been shown that the efficiency of silver nanoparticles compared to the ionic form (Ag +) indicates the presence of other nanoparticle nanoparticle removal techniques (16).

Another study of the treatment of pseudo-vesicular with 100 nanometers of nanoparticles led to a rapid reduction of biofilm up to 95%, which potentially reduced the effectiveness of silver particles (eg by retaining silver ions or local oxidation inhibition) (17), (18).

Studies on ag-ion inhibitory mechanisms on bacteria have shown that microorganisms that have been treated with Ag + have lost the ability to replicate DNA and have lost the function of some basic enzymes and cellular proteins. It has also been shown that Ag + connects important groups of proteins that in this study, we found that adding a high concentration of Ag + directly to biofilm has an effect on denaturization activity (19).

The study also showed that the effectiveness of silver treatment of nanoparticles largely depends on the bacterial cell wall. The synthesis, the appearance of synthesis of silver nanoparticles produces a tool that inhibits the growth of bacteria. These results show that nanoparticles of sizes 10 to 12 nanometers showed a much stronger antimicrobial effect other results indicate that gram negative bacteria are more susceptible to cell death than gram-positive bacteria due to their cell wall synthesis (20). It has also been shown that dehydrogenase activities in the respiratory tract *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been fixed by silver nanoparticles that can enter cells and pass through the outer membrane, Peptidoglycan, also states that silver nanoparticles can enter cells and damage the bacterial cell membrane, which inhibits the activity of some membrane enzymes and leads to cell death in both gram-negative and Gram-positive bacteria. Also increase the leaks of proteins and other molecules, and finally silver nanoparticles can inhibit ATPase, which plays an essential role in cellular metabolism, including bacterial growth (21) (22).

CONCLUSIONS

This study has shown that Silver nanoparticles have significant antibacterial effect on *Pseudomonas aeruginosa* regardless of it is susceptibility to antibiotics.

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