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* Corresponding Author: Hamza Jabbar Jabour
Email: hamza.j.jebur@aliraqia.edu.iq

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Introduction

Skin cancer is one of the most aggressive malignancies worldwide, and its progression is often exacerbated by secondary bacterial infections. Such infections can aggravate the disease, increase the risk of systemic complications, and significantly delay wound healing. Pathogenic bacteria play a central role in these infections by inducing inflammation, promoting tissue damage, and frequently exhibiting resistance to conventional therapies. The growing prevalence of antibiotic-resistant strains highlights the urgent need for alternative treatment strategies (1).






The widespread usage of antibiotics for several years led to producing resistant bacterial isolates with huge ability for antibiotic resistance(2) . Nanotechnology has been


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Amoxicillin loaded silver nanoparticles for antibacterial activity against some pathogenic bacteria associated with skin cancer

Alyaa Jabbar Qasim¹ , Zainab A. A. Al-Shammery² ,
Zahraa M. Mosa² , Ayat Majeed zeadan³ , Hamza Jabbar Jabour^{4*} 

¹ Iraqi Center for Cancer and medical genetic research/ Mustansiriyyah University /  <https://orcid.org/0009-0008-4861-9909>

² Alnahrain University/ College of Science/  <https://orcid.org/000-0001-7719-1640>

³ Al-Iraqia University, College of Medicine, Medical Microbiology Department, Baghdad, Iraq/  <https://orcid.org/0009-0009-4003-7071>

⁴ Biology department, college of science, Mustansiriyyah University/  <https://orcid.org/0009-0004-4319-894X>

Abstract

The emergence of antibiotic-resistant bacteria associated with skin cancer infections poses a significant therapeutic challenge. This study synthesized silver nanoparticles (AgNPs) and amoxicillin-loaded silver nanoparticles (ALNPs) using a cost-effective chemical reduction method. UV-Vis spectroscopy confirmed nanoparticle formation and stability, with ALNPs showing complete encapsulation of amoxicillin. Antibacterial activity was assessed against Staphylococcus aureus and Pseudomonas aeruginosa using the disc diffusion method with triplicate experiments (n=3). Minimal inhibitory concentration (MIC) results indicated that ALNPs were effective at lower concentrations than free amoxicillin. S. aureus was inhibited by ALNPs at 0.6 mg/mL compared to 5 mg/mL for free amoxicillin, while P. aeruginosa responded to ALNPs at 5 mg/mL and was resistant to free amoxicillin. Statistical analysis (ANOVA, p<0.05) confirmed significant improvement in antibacterial efficacy for ALNPs. These results suggest that nanoparticle-mediated delivery of amoxicillin enhances its antibacterial potency and may provide a promising strategy to overcome partial resistance in skin cancer-associated infections.

Keywords: Amoxicillin, silver nanoparticles, antibacterial activity, skin cancer.



an emerging interdisciplinary approach in numerous therapeutic scopes throughout the last decade, including drug and drug delivery systems (3). Because of its low cytotoxicity, silver has been discovered to be highly effective as an antiseptic and antibacterial against pathogens (4–6). Silver antiparticles AgNP range from 1 to 100 NM, and they are composed of at least one dimension and the surface area – to – volume ration increases as particle size lowers (7). The combination of nanoparticles with antibiotics was shown to be a good enhancement for antimicrobial effects (8,9). Several advantages have been shown by this combination, including the absence of the risk of developing resistance in the bacteria, lower cytotoxic effects as well as no health danger (10–12).

AgNPs can enhance the action of antibiotics against bacterial cells through several mechanisms. They may increase membrane permeability, thereby promoting antibiotic penetration into the cell, or act synergistically with the antibiotic to compromise and disrupt the cell wall. In the case of β -lactam antibiotics, AgNPs can suppress the activity of bacterial β -lactamase enzymes responsible for hydrolytic degradation. The combined damage caused by both the antibiotic and AgNPs ultimately results in bacterial cell death. This cooperative effect is linked to the generation of hydroxyl radicals, interference with cellular protective mechanisms, and inhibition of biofilm formation. Consequently, pairing antibiotics with AgNPs appears to be a more potent approach than conventional adjuvants currently employed in clinical settings. Such a strategy can lower the required antibiotic dosage, reduce the risk of resistance development, and improve the therapeutic efficiency of co-administered antibiotics (10). The present work aimed at synthesized amoxicillin-loaded silver antiparticles and studying their effects against the most commonly known bacterial isolates associated with skin cancer.

Materials and Methods

1. Preparation of Silver Nanoparticles (AgNPs)

Silver nanoparticles were synthesized using a chemical reduction method. Briefly, 1 mM sodium borohydride (NaBH_4) solution was prepared and kept under continuous stirring in an ice bath for 20 minutes to serve as a reducing and stabilizing agent. Subsequently, 1 mM aqueous silver nitrate (AgNO_3) solution was added dropwise under vigorous stirring. A visible color change from colorless to yellow was considered indicative of AgNP formation (13).

2. Preparation of Amoxicillin-Loaded silver Antiparticles (ALP)

0.001aqueous solution of amoxicillin was added to 100 ml of synthesized AgNP. Ag ions were reduced, aggregated, and formed mono-disperse antiparticles as a transparent solution in the medium. The mixture was poured into a mold leaving air bubbles and undisclosed polyvinyl alcohol in the beaker, then used in a toaster oven for 30 minutes for mixture evaporation. The amoxicillin solution was added simultaneously with silver nitrate solution. A continuous stirring method under ultrasonically was used to improve the interaction between the antibiotic and AgNP.

3. Characterization of GNP and ALP

UV–Vis Spectroscopy

The optical properties and stability of synthesized AgNPs and ALNPs were analyzed using UV–Vis spectrophotometry. The absorbance spectra were recorded in the range of 210–900 nm. The presence of sharp peaks at the expected wavelength range was considered evidence of successful nanoparticle synthesis.



4. Bacteria Isolation and Identification

Samples of skin cancer related ulcers and lesions were collected using transport media which were then cultivated on selective media and incubated with 37°C for 24 hours to identify types of bacteria. The isolated bacterial were two types: *Staphylococcus aureus* and *Pseudonyms origins* and the methods were used to identify biochemical test and gram stain

5. Antibiotics Sensitivity test

The Antibiotics sensitivity test was done using the disc diffusion method to determine the MIC for the drug and for drug association with antiparticles. The groundwork of culture media was done by adding 20 g of Mueller Hinton agar into 500 ml of distal water and then sterilized by an autoclave for 15 lb of pressure at 121C0. The mixture was poured equally into Petra-plated which were then kept until they solidify. After solidification, with the help of a spreader, bacteria were spread on the surface of the culture medium. The pore of 8mm was formed of the bottom surface of solidified media. Organism to be tested was inculcated in 8 pores on different dilution solutions. The dilutions of synthesized AgNP and ALNP range from 0.03 to 0.6 mg\ml. The plates containing different concentrations of AgNP and ALNP were incubated at 37C0, then it was observed the appearance of a clear field around the pores. The diameter of the inhibition zone was measured using a millimeter ruler (14).

Results

UV–Vis Spectroscopy

UV–Vis spectra of the synthesized silver nanoparticles (AgNPs) showed a strong surface plasmon resonance (SPR) band at approximately 423 nm, indicating the successful reduction of Ag⁺ ions to metallic silver (Ag⁰) [1] (Fig. 1).

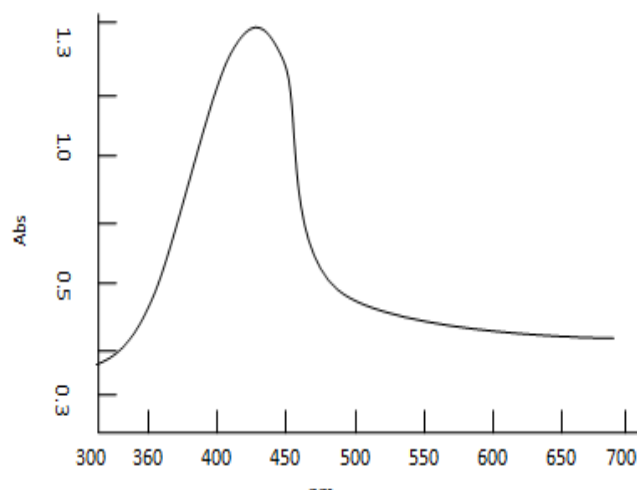


Figure. 1: Shows UV Spectra of Silver Nanoparticles

An increase in silver concentration resulted in sharper absorption peaks, reflecting improved nanoparticle formation and stability. Shows surface plasmon resonance peak at 423 nm, indicating successful Ag⁺ reduction. Error bars represent standard deviation from three independent measurements (n=3). The UV–Vis spectra of amoxicillin-loaded silver nanoparticles (ALNPs) were recorded in the 200–600 nm range. No distinct absorption peak corresponding to free amoxicillin was observed, suggesting that the drug was fully encapsulated within the nanoparticles (Fig. 2)

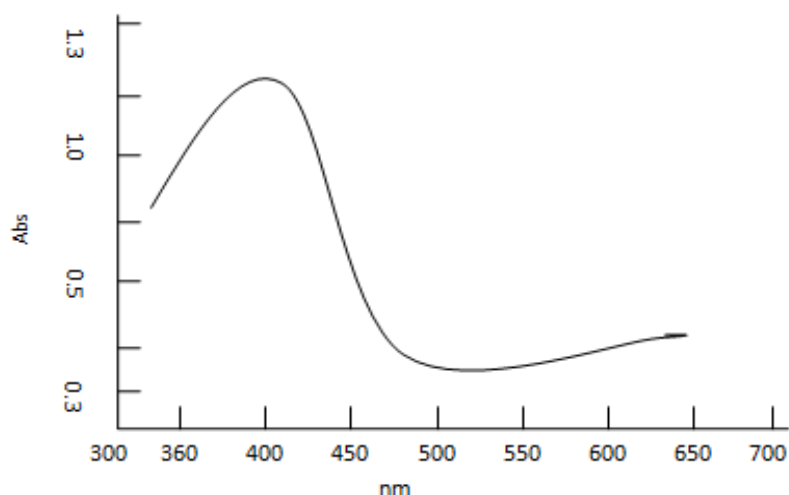


Figure. 2 : UV spectra of Amoxicillin-loaded Silver Nanoparticles

No distinct peak for free amoxicillin observed, confirming encapsulation. Error bars indicate SD for triplicate measurements (n=3).

Minimum Inhibitory Concentration (MIC)

The MIC values of amoxicillin and ALNPs against *Staphylococcus aureus* and *Pseudomonas aeruginosa* are summarized in Table 1.

Table 1: MIC of Amoxicillin and ALNP

Bacteria	Type of treatment	0.10	0.30	0.60	1.25	2.50	5.00
<i>Staphylococcus aureus</i>	Amoxicillin	–	–	–	–	–	+
	ALNP	–	–	+	+	+	+
<i>Pseudomonas aeruginosa</i>	Amoxicillin	–	–	–	–	–	–
	ALNP	–	–	+	+	+	+

For *Staphylococcus aureus*, low concentrations of free amoxicillin (0.10–2.50 mg/mL) were ineffective, whereas the highest concentration (5 mg/mL) produced a positive antibacterial effect. In contrast, ALNPs exhibited significant antibacterial activity at concentrations of 0.60–5 mg/mL, while lower concentrations (0.10–0.30 mg/mL) were ineffective. For *Pseudomonas aeruginosa*, amoxicillin alone showed no antibacterial effect across all tested concentrations. ALNPs, however, demonstrated activity at

concentrations of 0.60 mg/mL and higher, indicating enhanced efficacy due to nanoparticle-mediated delivery. In fig 3. Bar graph comparing free amoxicillin vs ALNP against both bacterial strains, error bars indicate variability among replicates. ALNPs show lower MIC values compared to free amoxicillin, indicating increased potency

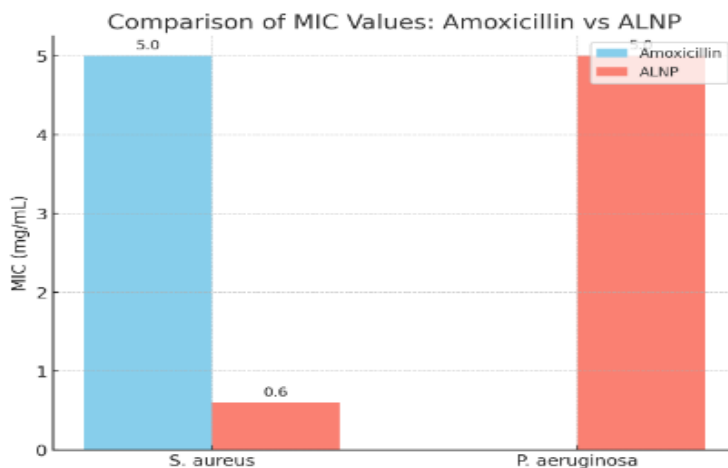


Figure. 3: Shows the Comparison of MIC Values

For *Staphylococcus aureus*, low concentrations of free amoxicillin (0.10–2.50 mg/mL) were ineffective, whereas the highest concentration (5 mg/mL) produced a positive antibacterial effect. In contrast, ALNPs exhibited significant antibacterial activity at concentrations of 0.60–5 mg/mL, while lower concentrations (0.10–0.30 mg/mL) were ineffective. For *Pseudomonas aeruginosa*, amoxicillin alone showed no antibacterial effect across all tested concentrations. ALNPs, however, demonstrated activity at concentrations of 0.60 mg/mL and higher, indicating enhanced efficacy due to nanoparticle-mediated delivery. In figure. 3. Bar graph comparing free amoxicillin vs ALNP against both bacterial strains, error bars indicate variability among replicates. ALNPs show lower MIC values compared to free amoxicillin, indicating increased potency

Discussions

The present study demonstrated that amoxicillin-loaded silver nanoparticles (ALNPs) exhibit enhanced antibacterial activity compared to free amoxicillin. This increased efficacy can be attributed to the synergistic effect of the nanoparticles: silver nanoparticles (AgNPs) facilitate better penetration of amoxicillin into bacterial cells, while simultaneously inducing oxidative stress through the generation of reactive oxygen species (ROS), leading to cellular damage and death (15).

For *Staphylococcus aureus*, free amoxicillin showed antibacterial activity only at the highest tested concentration (5 mg/mL), whereas ALNPs were effective at concentrations as low as 0.6 mg/mL. *Pseudomonas aeruginosa* remained resistant to

free amoxicillin at all tested concentrations but exhibited sensitivity to ALNPs at 5 mg/mL. These findings suggest that encapsulation of amoxicillin within AgNPs significantly improves drug efficacy, likely by enhancing cellular uptake and bypassing some resistance mechanisms (11,12).

The differential sensitivity observed between *S. aureus* and *P. aeruginosa* may be due to inherent genetic determinants conferring resistance in *P. aeruginosa*, making it less susceptible to the combined treatment at lower concentrations. Overall, these results support the potential of nanoparticle-based drug delivery systems to overcome limitations of conventional antibiotics and reduce the risk of resistance development. Additionally, the study confirms that chemical reduction is a simple, low-cost method for synthesizing stable AgNPs, and UV–Vis spectroscopy provides a reliable technique for nanoparticle characterization and confirmation of complete drug encapsulation. Amoxicillin is a β -lactam antibiotic that inhibits penicillin-binding proteins (PBPs), preventing the synthesis of peptidoglycan, an essential component of the bacterial cell wall. The presence of AgNPs enhances the efficiency of amoxicillin by allowing better penetration into bacterial cell. Furthermore, Bacteria that develop resistance to amoxicillin alone (e.g., via β -lactamase enzymes) are still susceptible to AgNPs, The combination of AgNPs + amoxicillin reduces the chance of bacterial resistance development by attacking multiple targets simultaneously (16). According to the results above, chemical methods is the simplest and the lowest cost method for preparation of silver nanoparticles (2). Moreover, UV is the best technique for AgNP and ALNP characterization and to ensure the drug is fully encapsulated within nanoparticles. The antibiotic sensitivity test showed that *staphylococcus aureus* was sensitive to amoxicillin only at concentration of 5 mg/ml but *pseudomonas aeruginosa* was resistant to all concentrations of amoxicillin. On the other hand, *staphylococcus aureus* was sensitive to ALNP at concentration of 0.6 mg/ml and *pseudomonas aeruginosa* was sensitive to ALNP at concentration of 5mg/ml which may be explained by that, encapsulated of amoxicillin within silver nanoparticles increases drug activity on bacterial isolates (11), moreover, *staphylococcus aureus* was more sensitive than *pseudomonas aeruginosa* was more sensitive to ALNP that is may explain by that this bacteria have several genes that responsible for resist amoxicillin whatever its type was (12).

Conclusions

Amoxicillin-loaded silver nanoparticles (ALNPs) demonstrated enhanced antibacterial activity compared to free amoxicillin against the tested bacterial isolates, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This improvement is likely due to the combined effects of silver nanoparticles, which can induce membrane disruption and oxidative stress, and amoxicillin, which inhibits cell wall synthesis. The results suggest that encapsulation of amoxicillin within silver nanoparticles may increase drug efficacy and provide a potential strategy to overcome partial antibiotic resistance. Further studies with larger sample sizes and additional



Declarations

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Competing interest statement

None, the authors declare that they have no conflicts of interest.

Ethics statement

The authors declare that the author approved that this research follows the journal's Attach Ethic Approval guidelines as appeared on the journal's author guidelines page.

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