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Study on the role of gold and silver nanoparticles on antibacterial activity and lung cancer cell line (A549)

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Abstract

Background: This study will evaluate gold and silver nanoparticles' antibacterial action against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and synergistic effects on the human lung epithelial cell line A549.

Methods: Gold and silver nanoparticles (33-40 nm) were obtained from Nanomaterials at quantities of 5, 15, 25, and 35 $\mu\text{g mL}^{-1}$. The University of Kerbala Biology Department donated bacterial isolates for this investigation. Clinical specimens yielded *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates. Vitek-2 confirmed the identities of the isolates and antibiotic susceptibility. Lung cancer was studied using A549 lung cancer cells from a 58-year-old Caucasian male's lung tissue.

Results: The results show that nano-gold complexes are more effective than silver nanoparticles against lung cancer. Gold nanoparticles exhibit a significant inhibitory concentration of 35 $\mu\text{g/ml}$, resulting in a 55% anticancer effect, while silver nanoparticles have a maximum inhibition of 49 $\mu\text{g/ml}$. At a concentration of 35 $\mu\text{g/ml}$, gold nanoparticles inhibit *Pseudomonas aeruginosa* with a 29 mm zone. No significant inhibition of cell growth was observed at lower dosages of 5, 15, and 25 $\mu\text{g/ml}$. The treatment's antioxidant and cytoprotective characteristics reduce paracetamol-induced oxidative stress, explaining this lack of difference. These findings imply that gold nanoparticles may protect against oxidative damage and cancer.

Conclusion: The cytotoxic effects of gold (AuNPs) and silver (AgNPs) on A549 human lung cancer cells were different. At 35 $\mu\text{g/mL}$, AuNPs inhibited cell growth by 55%, while AgNPs showed a 49% inhibition rate. AuNPs were more effective against *Pseudomonas aeruginosa* than *Staphylococcus aureus* than AgNPs. These findings show that AuNPs may be useful in anticancer and antibacterial therapy, depending on nanoparticle concentration and target specificity.



Introduction

Nanoscience and nanotechnology find extensive applications for metallic nanoparticles in many different sectors. In some cases, these nanoparticles could substitute often-used medications [1]. With distinct optical and electrical characteristics from conventional materials, emerging materials known as gold nanoparticles have great future use in the medical sector [2]. Gold nanoparticles are one of the rarest minerals on Earth. Moreover, it is considered to be one of the safest and is characterized as chemically inert [3]. One of the most often utilized nanomaterials is gold and studied nanoparticles, where gold metal is one of the most stable minerals, making it the most used in scientific research and study [4]. The production and synthesis of gold nanoparticles are subject to a bottom-up approach. Gold salts are reduced by the presence of stabilizing agents, which play a role in preventing the agglomeration of gold particles with each other [5]. The adjustable size of gold nanoparticles makes it simpler for them to pass through cellular membranes; it affects protein synthesis, metabolism, and cellular permeability, which results in bacterial cell death [6]. Silver (Ag) nanoparticle molecules are extremely small, with at least one dimension below 100 nanometers [7]. The researchers focused on nano-silver particles due to their thermal, electrical, and antimicrobial properties, such as a delivery, high thermal, electrical, chemical, catalytic, and counter-activity performance for microbes [8].

Staphylococcus aureus is a significant bacterium known for causing various inflammatory infections. This bacterium's presence in sewage, combined with its ability to produce various metabolites such as toxins and enzymes and form protective biofilms, contributes to its high level of antibiotic resistance and its ability to frequently infect humans [9]. Comparably, *Pseudomonas aeruginosa* is a well-documented antibiotic-resistant bacterium linked to several infections, including urinary tract ones. Its ability to build biofilm and the existence of antibiotic resistance genes—such as those present on plasmids (e.g., plasmid-B)—attribute to this resistance [10]. By offering a protective layer that helps these bacteria survive under hostile environments, Biofilms are crucial for enhancing their pathogenicity and antibiotic resistance. Furthermore, quorum sensing systems are essential for bacterial communication and survival in demanding conditions, hence supporting chronic and difficult-to-cure illnesses [11, 12]. The complicated and major illness known as cancer is typified by the unchecked growth of malignant tumors from cells. These tumors arise from genetic abnormalities that disturb normal cellular growth and control, hence producing aberrant tissue masses capable of invading

and damaging nearby tissues and organs [13]. Any portion of the body can have cancer; symptoms, course, diagnosis, and therapy differ greatly depending on the organ affected [14].

Methods

From nanomaterials, particle size (33–40 nm) gold and silver nanoparticles were obtained.

Bacterial isolates:

The Department of Biology, University of Kerbala laboratories provided two pathogenic bacterial isolates. Originally separated from clinical specimens, they were first diagnosed as *P. aeruginosa* and *S. aureus*; their confirming identification was obtained with the Vitek-2 method, which verified they were *S. aureus* and *P. aeruginosa*. These two isolates were utilized in the current investigation in addition to identifying their patterns of antibiotic susceptibility.

Antibiotic susceptibility tests of various classes of antibiotics were used in the antibiotic susceptibility test, which was carried out on bacterial isolates by the disk diffusion method (Kirby-Bauer method) as described by [15] and as per Clinical Laboratory Standard Institute recommendations. The sensitivity of antibiotics was determined by measuring the inhibition zone diameter. Using reference tables, the bacteria were categorized as resistant (R), intermediate (I), or sensitive (S) to the antibiotics.

Cell line:

Derived from basal epithelial cells of human lung tissue, the A549 cell line was developed in 1972 from lung tissue of a 58-year-old Caucasian man suffering with lung cancer. This cell line is frequently used as an in vitro model for lung cancer research and potential treatment testing.[16].

Thawing and culturing the cell lines:

The A549 cell line was thawed in a water bath at 37°C. Once thawed, the cells were distributed into culture flasks containing RPMI-1640 medium and allowed to adhere and proliferate for 24 hours in a humidified incubator set at 37°C with 5% CO₂. The growth conditions ensured optimal cellular proliferation and contamination checks. The cells were observed using an inverted microscope to confirm their viability, absence of contamination, and sufficient growth, reaching a density of 600-700 cells/microliter, equivalent to approximately 80% confluency. The cells were transported to a biosafety cabinet after they reached this confluency; the growing medium was taken out, and the adhering cells were thrice washed under PBS [17].

MMT test approach:

Detection of lung cancer cell line viability following gold and silver nanoparticle treatment.

-A method has been used in [18] the biology test as follows:

- Kit Contents MTT
- MTT 1 ml x 10 glass flasks
- Solubilization method Following a method for killing and preparing cell lines, cells were grown (1 x 10⁶ cells/mL). The cells were next coated with a sterile film on a 96-well flat-bottom plate. Each well's ultimate volume was 200 microliters.
- After 24 hours at 37°C at 5% CO₂, the medium was taken off of the plate. To the wells were specified quantities of silver and gold nanoparticles (5, 15, 25, and 35 µg/mL).
- Every concentration was tested thrice with a standard control.

- The plate was once more left at 37°C for 24 hours. Every well-received ten microliters of MTT solution (0.45 mg/mL).
- The plate was set at 37°C and incubated for four hours. Each well-received 100 microliters of solubilizing solution was added to gently dissolve the medium for five minutes.

- An ELISA reader at a 575 nm wavelength assessed absorbance.

- Applying the equation:
Viability % = (optical density of sample/control) × 100%

The statistically evaluated ELISA results showed the nanoparticle concentration needed to reduce cell line proliferation.

Statistical Analysis

Data were analyzed using statistical software (e.g., GraphPad Prism) to assess group differences. The t-test was used to compare means, with a P-value ≤ 0.05 considered statistically significant. Results were expressed as mean ± standard deviation (SD).

Results

With notable differences from the control, the present investigation revealed that silver and gold nanoparticles displayed antibacterial activity against *P. aeruginosa* and *S. aureus* isolates. Compared to the control, the present investigation indicated that silver and gold nanoparticles have antibacterial activity against *P. aeruginosa* and *S. aureus* isolates with extremely significant differences. The present results suggest that compared to *S. aureus*, gold nanoparticles demonstrated greater antibacterial efficacy against the bacterium *P. aeruginosa*. At different dosages of 35 µg mL⁻¹, the greatest rate of inhibition shows how well silver nanoparticles increase the inhibition of bacterial illness. A reduced inhibitory diameter was noted at 5 µg

mL⁻¹; *S. aureus* had a rate of 6mm whereas *P. aeruginosa* had an 8mm rate. The results showed that gold nanoparticles were efficient in reducing bacterial pathogenesis at different dosages, with the highest rate of inhibition at a concentration of 35 µg mL⁻¹ and a rate of inhibition diameter (29 and 24) mm for every bacterium and a lower diameter of inhibition at a concentration of 5 µg mL⁻¹. Anti-lung cancer medicines were treated with varying doses of nanoparticles (5, 15, 25, and 35 µg mL⁻¹). At an inhibitory concentration (35 µg mL⁻¹), A549 in a laboratory environment, gold nanoparticles showed greater anti-lung cancer activity than the silver nanoparticles, which came out to be 49 µg/ml. Values of suppression of cell growth at high concentrations (5, 15, and 25 µg mL⁻¹) showed no appreciable variations.

<i>S. aureus</i>	Antibiotics	<i>P. aeruginosa</i>	Antibiotics
resistant	Amikacin	resistant	Ciprofloxacin
resistant	Ceftazidime	resistant	Tetracycline
resistant	Meropenem	resistant	Vancomycin

Table 1: *Staphylococcus aureus* and *Pseudomonas aeruginosa* antibiotic susceptibility tests.

P-Value	Significance	gold nanoparticles		Silver nanoparticles		CON. (µg mL ⁻¹)
		<i>S. aureus</i> (mm)	<i>P. aeruginosa</i> (mm)	<i>S. aureus</i> (mm)	<i>P. aeruginosa</i> (mm)	
0.9657<	NS	11	18	6	8	5%
0.8776<	NS	14	20	12	18	15%
0.7655<	NS	19	23	16	20	25%
0.0061	**	24	29	20	24	35%

Table 2: The antibacterial activity of the silver nanoparticles. NS: Non-significant, **: P- Value >0.05, SD: Standard deviation.

CON µg mL ⁻¹	Percentage rate Vital after treatment. Silver nanoparticles% ±SD	Percentage rate Vital after treatment. gold nanoparticles% ±SD	significance	P- Value
%5	2.68±16	2.25±18	NS	0.9876<
%15	1.87±26	1.84±29	NS	0.9654<
%25	0.84±33	0.84±41	NS	0.9543<
%35	1.95±49	0.46±55	**	0.0025
	IC50: 2567.8	IC50: 1546.2		

Table 3: Bacterial survival rate after treatment with gold and silver nanoparticles with statistical significance values and IC50 NS : Non-significant ;**, P- Value , 0.05< SD: Standard deviation.

Discussion

The negative impact of industrial products used in various industries on human health is becoming more widely recognized, leading to a resurgence of interest in the characteristics of nanocomposites. Since ancient times, gold and silver nanoparticles have been valued as preferred inorganic antibacterial agents for preventing infections and spoilage. In the current investigation, gold and silver nanoparticles inhibited both Gram-negative and Gram-positive bacterial isolates, consistent with previous findings [19]. Several pathways contribute to this inhibitory effect. Gold nanoparticles, with their high penetrating power, target bacterial cell walls, cell membranes, proteins,

and DNA [20, 21]. Research shows gold nanoparticles can penetrate subcellular compartments and induce pits and cellular death. Furthermore, they can cause pit development by binding to the glycan ports of the cell wall and breaking the N-acetylglucosamine and N-acetylmuramic acid linkages in glycans. [22]. Beyond targeting cell walls and membranes, nanoparticles have been shown to affect other bacterial components, such as chromosomes and respiratory chain dehydrogenases [23]. This result aligns with findings reported by [24]. Significant reductions in the number of *S. aureus* and *P. aeruginosa* were observed following treatment with gold nanoparticles compared to the control.

Development of cancer treatment based on nanotechnology aims to reduce adverse effects connected with conventional medications. Many traditional treatments are useless against cancer cells since they show flaws in the pathways of programmed cell death (apoptosis). Two main goals of nano therapy are to overcome drug resistance, a major barrier in cancer treatment, and improve the therapeutic efficacy of medications.

Several laboratory studies (in vitro) have highlighted the mechanism of action of silver nanoparticles, which allows them to enter cells via the cellular absorption process and localize within the cytoplasmic space and compartment. Additionally, silver nanoparticles can penetrate mitochondria, the energy stores of the cell, and disrupt the respiratory chain, leading to the production of oxygen free radicals. This mechanism sets off programmed cell death, defined by cellular shrinkage, DNA fragmentation and membrane blebbing, which finally results in the regulated elimination of damaged or undesired cells [26]. This finding aligns with [27], which noted the effect of silver nanoparticles at a concentration of 7 mg/mm on colon cancer cells. Furthermore, [28] demonstrated that the anticancer properties of nano-propolis extract are attributed to the presence of flavonoids, which inhibit the cancer cell cycle, proliferation, and tumor growth, thereby causing cell cycle arrest and apoptosis. This study highlighted propolis nanoparticles as a viable alternative for cancer treatment. The results imply that different dosage formulations for the treatment of major diseases, including cancer [29, 30, 31, 32] could be developed using nano-red propolis extract. The application of various concentrations of gold and silver nanoparticles demonstrated significant inhibitory activity against human lung cancer cells (A549). Gold nanoparticles exhibited an inhibitory effect of up to 55%, while silver nanoparticles showed an inhibitory effect of 49% at a concentration of 35 µg/mL. Additionally, gold nanoparticles proved to be more effective as antibacterial agents compared to silver nanoparticles, with stronger antibacterial action

observed against *Pseudomonas aeruginosa* than against *Staphylococcus aureus*.

Author Contributions

N.A. (Noor Alkharsan) helped design, analyze, and interpret the study. Zainab Naser Al-Laith oversaw technique and data gathering. S.Z.A. was involved in manuscript drafting and literature review. N.A.-I. (Nibras Al-Ibrahem) revised the article and verified data. All writers read and approved the final article.

Conflict of Interest

The writers identify no conflict of interest.

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