

Gold Nanoparticles and Laser as Antimicrobial Agents against Some Types of Bacteria

Maysa S.M. Al-Shukri

Department of Microbiology, College of Medicine, Babylon University, Hilla, Iraq.

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The goal of this study was to assess the efficacy of gold nanoparticles (AuNPs) and AuNPs– Low level laser combined therapy as antibacterial agents against *different types of bacterial isolates in vitro*. Five concentrations of AuNPs (25,50,75, 100 and 200 µg/mL) were employed for assessing bacterial growth rate and the bacterial MIC. It was found that there was slight reduce in bacterial growth after treatment with gold nanoparticle. The influence of gold nanoparticles on *Staphylococcus aureus* biofilms was also studied. It was found that biofilm growth was decreased at different concentrations of AuNPs compared to absence of nanoparticles. Results of the cytotoxic act of AuNPs and low level laser effect in combination against *Escherichia coli* and *S. aureus* referred that the low level laser light improved the antibacterial action of AuNPs when it used in combination with low level laser.

Keywords: Gold nanoparticles, Laser, Antibacterial agent, Cytotoxicity, MIC.

Nano materials become a favorable and effective candidate that can replace standards resources with maximum uses in all areas of technology and science, due to their ultra-small size of that have higher ratio of surface area to volume (SA/V ratio) and at their outer surfaces, prolong the number of active atoms, like silver, gold and zinc, each with diverse features and wide range of activities¹.

Among NPs, AuNPs are many that applied as a catalyst for gene therapy, medical therapy and biological diagnostic methods². The important benefit of AuNPs is that they are simple to be made by the chemical reduction methods and they have a small level of toxicity when compared with others nanomaterials. Many element methods have been assumed for different dimensions of nanoparticles and to organize their surface to make the uses to be increased³.

The development of multi-drug resistant (MDR) bacteria and their effect on human health inspires investigators to create new methods to improve active antibacterial agents to conquer the bacterial drug resistance and diminish their cost⁴. One method being recently assessed is photodynamic therapy (PDT), which employs dyes that absorb light to make toxic oxygen radicals to destroy the bacterial cells. Nevertheless, this management may be not active for bacterial infections in hypoxic environments. An additional talented method is to practice metal nanoparticles, and laser energy to kill the microbes through Photo Thermal Therapy (PTT)⁵. Low level laser have medical applications such as bio-stimulation, destructive and inhibitory effects⁶, Laser therapy is considered as a non-invasive treatment method with several special effects and applications in clinical work, including stages of tissue healing⁷.

Optical characteristics of conductive NPs, like those made of gold have been connected with Surface Plasmon Resonance (SPR), which as soon as restricted in tiny colloids, is mentioned as localized surface Plasmon resonance (LSPR).

* To whom all correspondence should be addressed.
Tel.: +9647801084642;
E-mail: maysaalsukri@gmail.com

This results in wavelength dependent photo thermal effect, in which the electrons oscillate cooperatively when irradiated with specific light energy resonated with its LSPR.

When gold nanoparticles absorb light energy, they emit heat in conformity with. The releasing of such heat makes gold nanoparticles suitable in different photo thermal therapy applications like those directed against bacterial and cancer cells. The death of the bacterial cells is happened as a result to physical disruption of these cells induced by Laser photothermal phenomenon⁸.

This study aims to detect the effectiveness of gold nanoparticles and AuNPs–laser induced therapeutic approach (at different energy densities of laser and different concentrations of AuNPs) as antibacterial agents against some pathogenic bacterial strains.

MATERIALS AND METHODS

Synthesis of the gold nanoparticles

Gold nanoparticles were produced as a solution by chemical procedure were 3 mM HAuCl₄ solution was reduced by the use of 10 mM NaBH₄ solution under continuous stirring. For additional and complete reduction of the mixture, the mixture was reduced again by 10 mg/ml solution of dextrose. The mixture was exposed to continuous stirring. After that, the mixture was washed numerous times with methanol using centrifugation at 65,000 rpm, AuNPs samples were characterized by using TEM. The size range of gold nanoparticles was 20-30 nm⁹.

Bacterial Strains

The procedure for antibacterial effect of AuNPs was made by using some types of bacterial isolates (*E. coli* and *S. aureus*) which were supplied by the Department of Microbiology, University of Babylon, collage of medicine, Iraq.

Detection of Antimicrobial Activities of AuNPs

An aliquot of 20 mL from readily prepared culture medium (holding 10⁵ CFU/mL of bacterial cells) was liquated and dispensed into test tubes, then a volume of 50 μL of these solutions was put into a 96-well plate and mixed with 50 μL of NP solutions in M9, making a final bacterial concentration of 5×10⁵ cfu/mL. NPs concentration differs ranging from (25, 50, 75,100, and 200 μg/

mL). A growth control group without NPs and a sterile control group with only growth medium were carried out at the same time. For estimation of the inhibition zone and measuring the diameter of inhibitory zones in mm, the method of Shamaila *et al.*³ was followed.

Effect of Nanoparticles on Bacterial Biofilms

Staphylococcus aureus and *E. coli* were used for this test. Bacterial biofilm formed in polystyrene microtiter plate overnight cultured bacterial cell were suspended with medium containing AuNPs at different concentrations, ranging as (25, 50, 75,100, and 200 μg/mL). 100μl of the suspension were add in to 96–well plates. The plate covered with lid and incubated at 37° C for 24 hr. After that the plate were wash with PBS and go to next steps which included the crystal violet staining¹⁰.

Analysis of Biofilm Formation with the Crystal Violet Staining

The method of crystal violet staining¹¹ was done for detection of the effect of nanoparticles on the production of bacterial biofilm.

Experimental Design of Laser Irradiation

Bacterial cells were exposed to a diode laser, at a wavelength of about 532 nm, outpower of 220 mW in a continuous type of wave. The irradiation with 532 nm light of laser was made according to standard methods^{12,13} at the doses of 3.9 ,7.8, 11.6 and 15.6 J/ cm² in term of deposit energy. The treated bacteria were separated into five groups: the group 1 as a control (not irradiated); group 2 (3.9 J/cm²); group 3 (7.8 J/cm²); group 4 (11.6 J/cm²), and group 5 (15.6J/cm²)⁶.

Antibacterial effect of Laser-induced AuNPs

The MIC for two treated bacteria groups was carried out from a mixture of bacterial culture and AuNPs (25, 50, 75,100 and 200 μg/mL) and the culture tubes were incubated at a degree of 37°C in shaking incubator for 24 hours.

Earlier to incubation, the culture tubes were exposed to 532 nm laser light, 220 mW, at doses 15.6 J/ cm² for each group as consider the best dose, correspondingly. An aliquot of 50 μL from each culture tube was spreader onto nutrient agar and incubated at a degree of 37°C for 24 h; the numbers of bacterial colonies grown on agar plates were counted. The measurements of optical density at 590 nm of treated bacteria groups were graphed for estimation of the bacterial growth curves¹⁴⁻¹⁵.

RESULTS

The Effects of gold nanoparticles on both Gram-negative and -positive bacteria are study, Bacterial strains were cultured with (25, 50, 75, 100 and 200 $\mu\text{g}/\text{mL}$) AuNPs and incubate at 37°C while untreated bacteria was used as a negative control, the OD590 values were monitored from each bacterial strain (*E. coli* and *S. aureus*) to each concentration of nanoparticle and the results showed that the influence of the AuNPs on different bacterial growth. The presence of AuNPs slightly inhibited bacterial growth (Figure 1).

In vitro test results reveal significant inhibitory effect on bacterial biofilm production in the presence of the gold nanoparticles. The effect were depend the type of bacteria, nanoparticle, and

concentrations of nanoparticle. At a concentration 75, 100 and 200 $\mu\text{g}/\text{mL}$, gold nanoparticles effect showed a reduction of about (13%) in *S. aureus* biofilm production, whereas in *E. coli* the nanoparticles showed no effect on biofilm production compared to control. Interestingly, at higher concentrations (75,100 and 200 $\mu\text{g}/\text{mL}$) a significant inhibition in biofilm production (*S. aureus*) was observed with the presence of gold nanoparticles when compared to its with low concentration (Figure 2).

The study also showed an inhibitory effect of 532nm diode laser on bacterial growth alone (Figure 3) and in combination with different concentration of gold nanoparticles for dose of 15.6 J/cm^2 . While the results revealed that *S. aureus* and *E. coli* showed no significant difference in growth

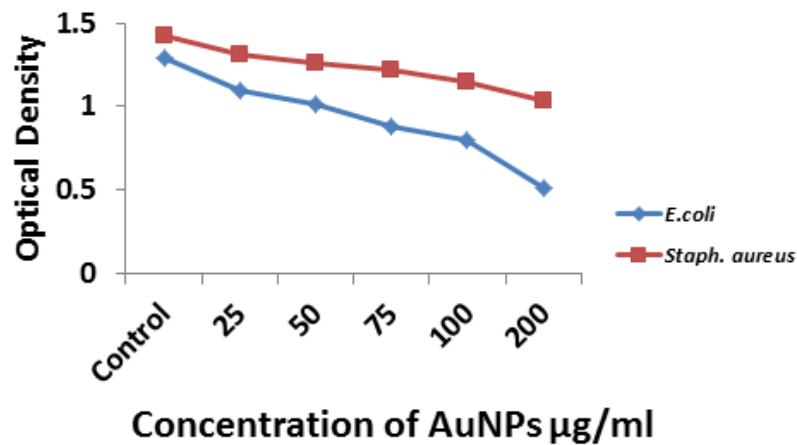


Fig. 1. Effect of Different Concentrations of AuNPs on the Growth of Bacterial Isolates

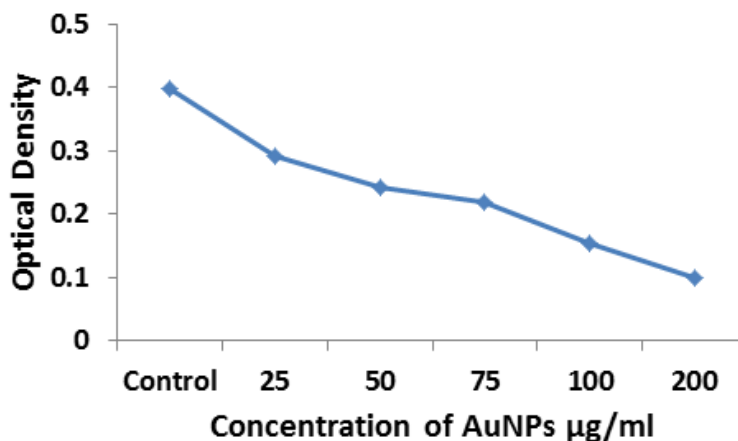


Fig. 2. Effect of Different Concentrations of AuNPs on the *Staph aureus* Biofilm Formation

when irradiated with the 532nm diode laser alone when exposed to different doses (3.9,7.8, and 11.6) J/cm² respectively.

The MIC of AuNPs-laser induced was estimated, and it revealed a relative study between applying of AuNPs alone as antibacterial agent and AuNPs-laser combined therapy. Laser enhancement it is a dose depended. The optimal dose was 15.6 J/cm². It was obviously recorded in the equivalent growth curve (Figure 4).

DISCUSSION

Nano-methods had a considered worldwide consideration due the fact that the nanoparticles have distinctive and novel features

from their bulk equivalents. The antimicrobial effects of the different nanoparticles had been widely reported by several authors with various and important Gram positive and Gram negative pathogenic bacteria like *E. coli* and *S. aureus*¹⁰.

Chatterjee *et al.*¹⁶ found that when cultures of *E. coli* isolates were treated with several concentrations of AuNPs as (25 µg/mL, 50 µg/mL, 75 µg/mL and 100 µg/mL), there was no significant effect in the curve of growth. The experiment of bacterial growth under the influence of the gold nanoparticle, reveals the nontoxic nature of these nanoparticle in the bacterial organism (*E. coli*). Therefore, it can be applied in the biological uses with the least probabilities of cytotoxicity.

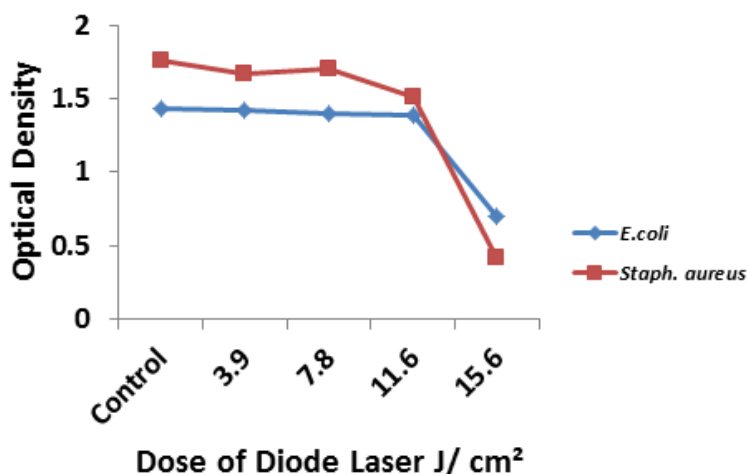


Fig. 3. Effect of Different Doses of Diode Laser on the Growth of Bacterial Isolates

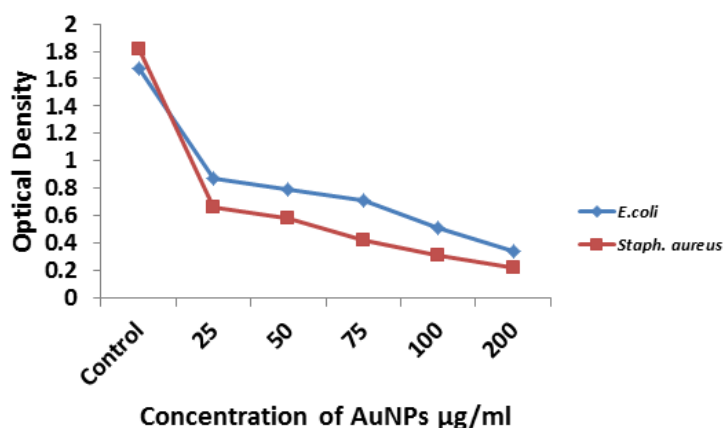


Fig. 4. Effect of Combinations Different Concentrations of AuNPs and LASER (for 20 min) on the Growth of Bacterial Isolates

The bactericidal activity of AuNPs against different pathogenic bacterial strains of Gram Gram negative bacteria like *Salmonella typhi*, *Pseudomonas aeruginosa*, *E. coli*, and *Klebsiella pneumoniae*, are vary, where MICs fluctuated from 20 to 40 µg/mL. This may be due to the fact that thicker cell wall of bacteria will diminish diffusion rate of these nanoparticle through cell wall (at lower concentrations) and subsequently reducing the effective action of gold nanoparticle as antibacterial agents¹.

AuNPs have been reported to aggregate on and react with both Gram- negative and Gram-positive bacterial cell membranes, with the inhibition of protein synthesis of bacteria and to prevent synthesis of the cell membranes¹⁷.

Shi *et al.*¹⁷ found that the biofilm production of bacteria increased quickly at the first 12 hours with the absence of the gold nanoparticles. This increment was inhibited by the nanoparticles at A 6 hours and more significant at 6 to 12 hours with the decrement in bacteria numbers in the biofilm. These results recommended that these nanoparticles are significantly effective in restriction of bacterial colonization in the biofilm.

Several author worldwide who studied the bactericidal effect of laser radiation reported that the radiation absorbed by chromophores may result in changes in molecules conformation, creating free radicals and reactive oxygen which, subsequently, stimulate disturbance in the structures of bacterial and fungal membranes¹⁸.

It was reported that the laser light increases the antibacterial effect of AuNPs by as a minimum of one fold. This may be referred back to the fact that photo thermal effect caused by the exclusive character of nano scaled gold colloidal liquid mixture that has robust boosted absorption band at 524 nm equivalent to the AuNPs SPR oscillations. When subjecting to resonating laser emission line, the kinetic energy of AuNPs increases¹⁹.

The created photothermal consequence can be active for fast and effective bacterial cell destruction⁸. The laser enhancement of the antibacterial action of AuNPs relays upon the exposure time at which the bacterial cells are treated. These results were corroborated by a previous study that investigated the antibacterial effect of AuNPs-laser, this effect was enhanced by

photothermal degeneration in combined approach, AuNPs-laser, results in rapid loss of bacterial cell membrane integrity²⁰.

CONCLUSION

AuNPs have been found to have a vital and effective revolution for drug delivery. Also, they perform as a non-dangerous and non-toxic antimicrobial agent refers to their functional effective nature when compared with antibiotics. This study can conclude that AuNPs united with laser exposure could be employed as confined effective antibacterial because that low level laser increases the antibacterial action of AuNPs by as a minimum of one fold.

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