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# Antibacterial Effect of Silver Nanoparticles on Pseudomonas Aeruginosa Bacteria

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**Abstract:** Silver nanoparticles(AgNPs)can simply synthesized by laser ablation. It play a significant role in the field of physics, biology, and medicine. The synthesized silver nanoparticles were confirmed by visual observation, optical spectroscopy, and TEM analysis. The synthesized AgNPs were tested for antibacterial activity against *Pseudomonas* microplate assay and agar well diffusion method. The AgNPs at 1 $\mu gml^{-1}$ and above showed noticeable antibacterial action for mainly following long incubation periods. In order to inspect the synergetic effect of laser light with AgNPs, 405 nm laser light (10mW) was used to irradiate bacterial suspension containing (16 $\mu gml^{-1}$) of AgNPs. Laser light elevates the cytotoxicity of nanoparticles.
**Keywords :** silver nanoparticles, antibacterial activity, nanoparticles-laser synergism.

## Introduction

Nanoscale particles show unique physiochemical properties, and attracted a lot of attention to the distinct characteristics. One of the distinctive characteristics is the ratio of surface area to volume [1-3].

For this and other reasons, the importance of nanotechnology is grows and find many applications in biological, physical, medical and others were found[4-8].bacterial resistance to antibacterial material and antimicrobial agents has been increased in recent years[8].Nanoparticles interact with biomolecules and microorganisms and therefore, there is an expansion in the field of research in this area. It has been known for centuries that silver and their compounds show antibacterial activity.There are many areas in which silver nanoparticles had been proved to be effective. As well as, there are a number of applications that use silver nanoparticles was made successfully as catalysts for many reactions[4].Here, we try to use their antimicrobial characteristics in inhibiting the bacterial growth or killing it[6-9].

Some of the physical properties of the particles of silver nanoparticles AgNPs, such as size (surface area), shape, surface charge and the speed of melting, is particularly important to determine the interactions and biological effects. Small particles have a larger surface area and, therefore, have a greater possibility of toxic. It was known that the silver nanostructure can dramatically affected on the physical and chemical nanoparticle properties. Utilized silver nanostructures in the biomedical field include silver spherical nanoparticles, nanowires, nanorods, nanoplates, and nanotubes[10,11].

Interaction of the nanoparticles with the bacterial surface cause changing of the membrane properties. The small size and very large surface area of nanoparticles allows them to make adhesion with the microorganism's surface, also small size of nanoparticles show best antibacterial activity, anddue to its tiny size, it can easily penetrate the cell and reach the inner contents of bacteria[6,12,13].

The nanoparticles clump to form geometric forms has been shown the conglomerate AgNPs happens in culture media, the cytoplasm and nuclei of cells, and that the dissolution of AgNPs comes as a result of surface oxidation, which leads to formation of the silver ion $Ag^+$ which can interact with Sulphur-containing proteins in the bacterial cell wall, which may lead to hazard functionality. This state is often considered as the main mechanism of the agent activity of silver nanoparticles. Dissolution depends on the chemical and surface properties of particles as well as its size, and is affected as well as the circumstances surrounding. Also nanoparticles toxicity also depends upon the pH and concentration of the nanoparticle interruption and toxicity is produced by the inherent properties of the silver nanoparticles[6,14,15]. Laser light reveals many applications in the field of bacteriology and nanotechnology. A few recent studies concerned with the activation of nanoparticles by visible laser light to enhance their antibacterial action[16]. Near UV laser had a visible bactericidal effects on many types of bacteria[17].In this work, we tried to elucidate the synergic effect of near UV laser light and silver nanoparticles on the viability of *pseudomonas aeruginosa.*

## Experimental

### Synthesis and Characterization of silver nanoparticles

Silver nanoparticles were prepared in the laboratory using (Laser ablation method). Briefly, 2 g of highly purified (99.99 %) silver plate was used as a target metal. The surface of the silver plate was polishes and cleaned with distilled water to remove any impurities, then cut into 1x1 cm pieces. These silver pieces were placed in the bottom of quartz cell containing 2 ml of distilled water. Laser ablation process was performed using aQ-switched Nd: YAG laser witha wavelength of 1064nm,a frequency of (5) Hz , pulse width of (7) ns and pulse power of (700) mJ. The number of pulses used were (30) pulses.This procedure produces silver nanoparticle solution with concentration of 50 PPM in distilled water.

Silver nanoparticle solution was characterized using UV-visible spectrophotometer(CECIL CE 7200,ENGLAND), scanning electron microscopy (SEM)(Model: LEO 1450 VP , voltage : 20 kv, Germany) and transmission electron microscopy (TEM)(Model: LEO 912 AB, Germany).

### Bacteria

*Pseudomonas aeruginosa* isolated from patients with superficial wounds in Hilla surgical hospital. Swaps of these wounds were transferred using sterile screw caps tube containing nutrient broth. Broths were then streaked on nutrient agar plates and incubated at $37C^0$ for 24 hours in the incubator. Characterization and identification of *Pseudomonas aeruginosa* was carried out depending on the morphological aspects and biochemical tests. This gram negative bacteria was used as a target to evaluate the antibacterial effect of silver nanoparticles. Bacterial cells were suspended and diluted using nutrient broth to $10^{-5}$ dilution which was equivalent to bacterial concentration of approximately $10^6$ cells/ml, confirmed via plate counts method. Bacteria were treated with different concentration of silver nanoparticles ranged from 0.5 up to 8 $\mu g ml^{-1}$.

### Laser

Cw semiconductor laser (405 nm), 10 mW output power was used for irradiating the bacterial suspension.

### Antibacterial activity

Bacterial growth inhibition was studied using optical method according to Amin *et al*, 2009 method with some modification[8]. Briefly, bacterial suspension was activated though incubating the bacterial suspension overnight in nutrient broth, then 1 ml inoculum was mixed with 9 ml of Nutrient broth (NB). Serial dilutions were prepared from this broth, 200 µl from the $5^{th}$ dilution was transferred to each sterile well of 96-well plate. Polystyrene 96-well flat-bottom tissue culture plates were used in this study. From the stock of silver

nanoparticles, different volumes were added to each well until it reach the required final concentration. Growth profiles in the 96-well plate were monitored (as OD) with a microplate reader (Huma Reader HS, Japan). OD was measured at a wavelength of 405 nm since bacterial suspension has high absorbance at UV region (figure 3). Samples were analyzed in replicates at room temperature with shaking before each measurement. The data was recorded after 0,1,2,3, and 24 hours.

Agar wells diffusion method was used to confirm these effects. In this method, Nutrient agar plate seeded with the bacteria and drilled with a sterile corkborer to make holes with diameter of 6 mm. These holes filled with 100 µl of AgNPs at different concentrations and the plate were incubated at 37 $C^0$ for 24 hours. Next day, inhibition zones were measured using ruler[18].

The effect of laser light on the viability of the bacteria in the presence of AgNPs was performed by irradiating bacterial samples containing (16 $\mu gml^{-1}$) of AgNPs for 1, 3, 5, 10, and 20 minutes and the effect was monitored after 1, 2, and 24 hours.

## Results and Discussion

### Characteristics of synthesized AgNPs

Silver (Ag) nanoparticles solution appears with a dark yellow color as shown in Fig. 1 (B). UV-Visible spectrophotometric analysis of silver nanoparticles is shown in fig.1 (A) where the peak absorbance of AgNPs was at (420-425) nm.

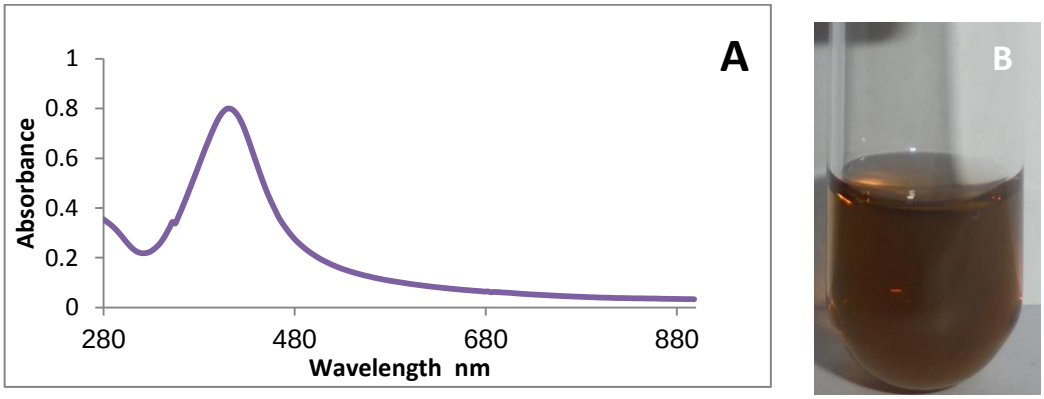


**Figure 1: Characteristics of silver nanoparticles. (A) UV-Visible spectra of silver nanoparticles (B) shows the dark yellow color of silver nanoparticles solution**

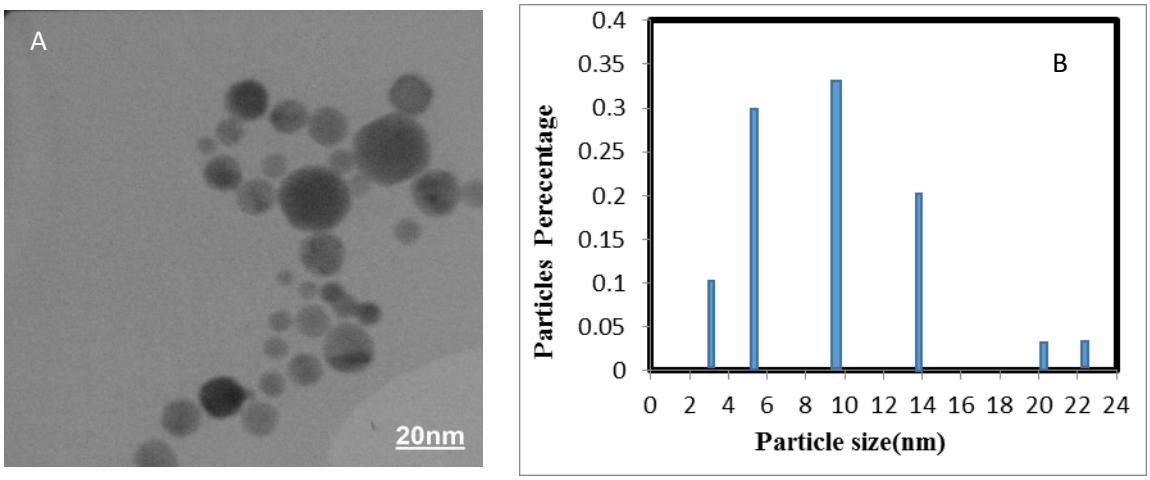


**Figure 2: Silver nanoparticles dimensions.(A) TEM images of Ag NPs. (B)shows the particle-size distribution.**

Our results are in agree with those reported by (Savithramma N*et al*,2011)(Sanchez-Ramirez JF*et al*,2008)(Leena F Hamza*et al*,2014). In summary, AgNPs were synthesized through citrate reduction of mixed metal ions of low concentrations or by using Medicinal Plants.

**Antibacterial activity of AgNPs**

Depending on the optical properties of bacterial suspension, optical measurement is used to determine the bacterial growth profile and follow up the effect of AgNPs effect on bacterial cells. Figure 3 illustrates the absorption spectrum of bacterial suspension, bacteria has a high absorption coefficient at the ultraviolet region. For this reason,the wavelength 405 nm was used to record the absorption of the same photo absorbing molecules that will be activated by 405 nm laser light. Since AgNPs has a relatively high absorption in this region, the measured OD is related to the bacterial and AgNPs absorption, but the change in OD will be restricted to the change in bacterial population, since AgNPs are stable and its numbers are constant.

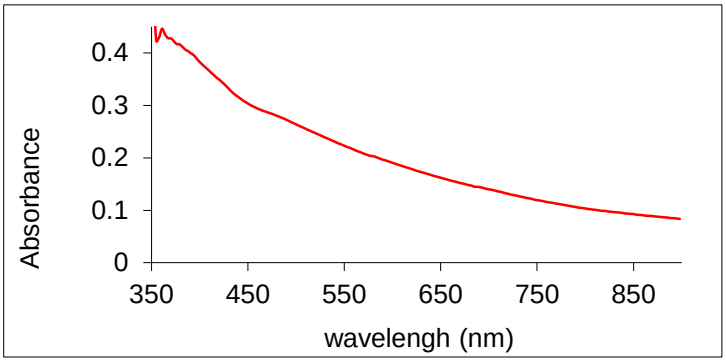


**Figure 3: absorption spectrum of bacterial suspension. Pure nutrient agar is used as blank for calibration**

For screening the antibacterial effect of AgNPs, different concentrations of AgNPs (0.5, 1, 2, 4, 8, and 16) µgml$^{-1}$have been monitored at different times (0, 1, 2, 3, and 24) hours. By observing the results, the bacterial inhibition is directly proportional to the concentration of silver nanoparticles, figure 4shows a clear variation in bacterial inhibition when using nanoparticles with concentrations (0.5)µgml$^{-1}$ and (16) µgml$^{-1}$ respectively. Table (1) shows the same results obtained by agar well diffusion method. Here, the cytotoxic effects appear more lucidly than optical method as well as disc diffusion method used in other studies[21,22]. In fact, this method is the most accurate, sensitive, andrapid tool to examine the antibacterial efficiency[23]. When the nanoparticles concentration was increased, the amount of active oxygen species produced (singlet oxygen and other free radicals) and thus increases the rate of bacterial inhibition. Several studies have noted the adhesion and accumulation of AgNPs to the bacterial surface creating gaps in the integrity of the membrane bilayer which cause an irreversible increased of cell membrane permeability leading to the formation of permeable pits. Thiscause an osmotic collapse in the bacterial cells and discharges the intracellular constituents[24]. In addition, Ag ions bind to the negatively charged protein and nucleic acid, causing deformations in the wall, membranes, and in the bacterial nucleic acids. silver ions also interact with a number functional groups such as thiols, phosphates, hydroxyls, imidazoles and indoles[25].

Few studies suggest that AgNPs have less effect on Gram-positive bacteria than Gram-negative bacteria due to the difference in the composition of the cell walls[25]. Other studies have shown that there is no significant difference for the impact of silver nanoparticles on bacteria whether grams positive or negative. These studies showed that AgNPs cause general fetal effects on both types of bacteria including; alteration of membrane permiability, DNA damage, and interaction of Ag+ ions with sulphur-containing proteins leading them to loss their activity. The effective bacterial killing of silver nanoparticles depends on their stability nature in the culture medium, to offer enough time for the interaction of the bacteria with silver nanoparticles[13,26,27,28].

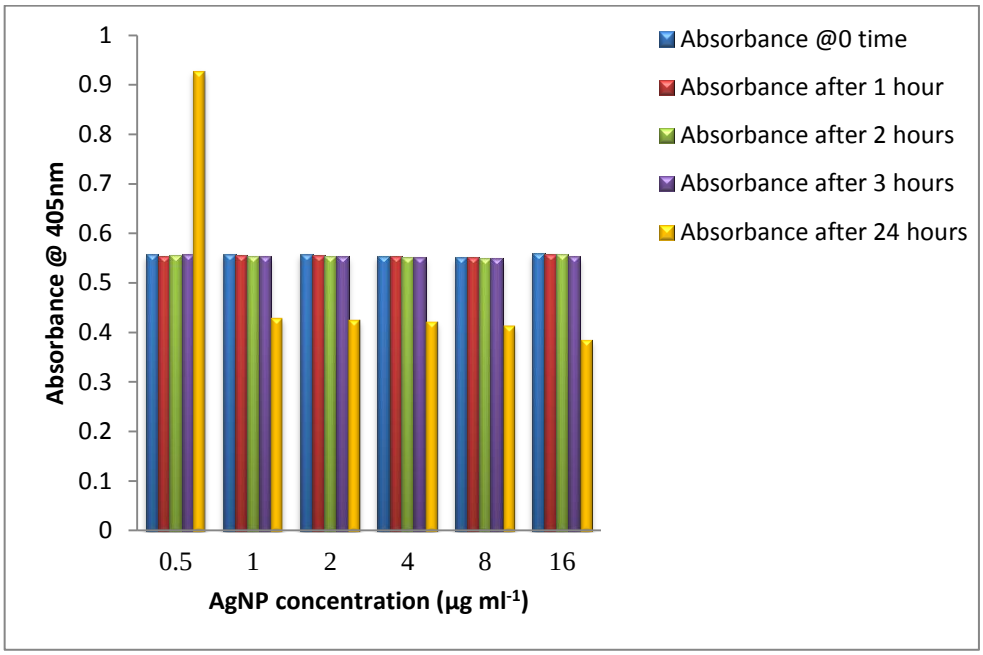


**Figure 4: shows the antibacterial effects of different concentrations of silver nanoparticles on *Pseudomonas aeruginosa***

**Table 1: Effect of different concentrations of AgNPs on the viability of *Pseudomonas aeruginosa* using agar well diffusion method**

| Concentration of AgNPS ($\mu gml^{-1}$) | Inhibition zone (mm) |
|---|---|
| 0.5 | 6 |
| 1 | 7 |
| 2 | 9 |
| 4 | 11 |
| 8 | 17 |
| 16 | 22 |

In other studies, AgNPs caused significant bacterial killing within shorter times. This variation in action is correlated to the difference in particle size. It is confirmed that the cytotoxicity of AgNPs is inversely proportional to its size[29,30].

**Effect of laser light on bacterial growth**

Semiconductor laser with a wavelength of 405 nm and power of 10 mW was used access the effect of laser light on the viability of *Pseudomonas aeruginosa* in the presence or absence of AgNPs. Various irradiation times (0, 1, 3, 5, 10, and 20) minutes were experienced. Growth profile was examined after 0, 1, 2, 3, and 24 hours as shown in figure 5. No significant alteration in growth profile was detected for all the irradiation times. Previous studies showed that 405 nm laser affect bacterial cells in amode similar to visible light with wavelength greater than 400 nm. It stimulates the endogenous porphyrins leads to the release of cytotoxic reactive species especially ROS[31]. The energy density used here ranged from 9.34-186.8 J/cm$^2$, these doses were reported to be ineffective against $G^{-ve}$ bacteria due to lower photon energy of this laser. In fact, irradiation with low intensity near UV or visible light may promote bacterial growth by generating low quantities of ROS that is required to induce cell growth[32,33].

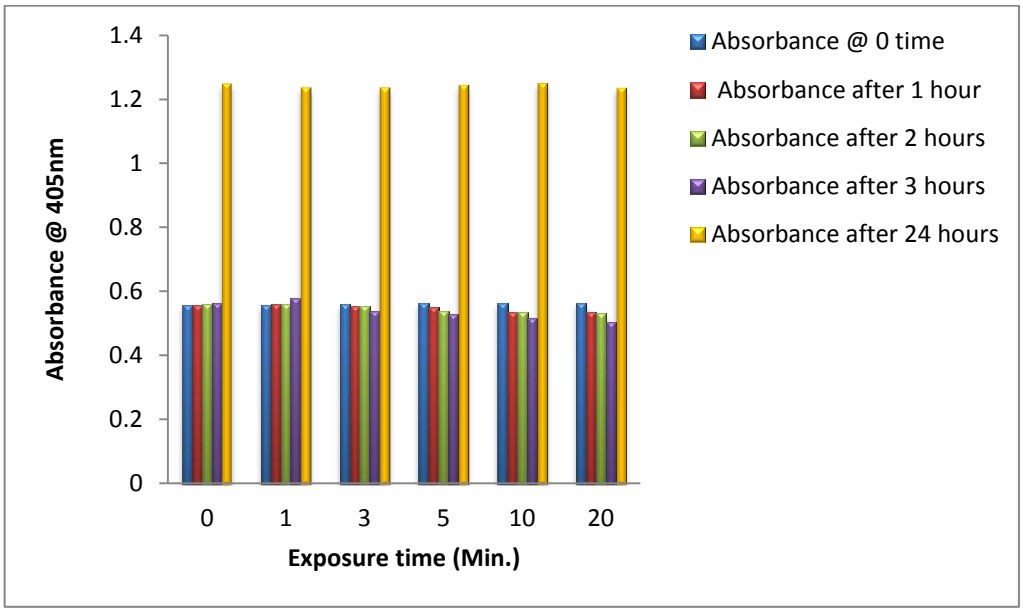


**Figure 5: Effect of 405 nm laser light at deferent exposure times on the viability of *Pseudomonas aeruginosa*. No significant changes were observed for all exposure times.**

The next step was to evaluate the effect of laser light in the presence of AgNPs in the bacterial culture media. As illustrated in figure (6), there was no clear difference in bacterial inhibition at times between 0-3hours, but at longer incubation times (24 h), the synergic cytotoxicity was obvious. This may due to direct proportionality between the laser dose delivered to the samples and the quantity of ROS generated. The changes in the bacterial number detected as OD is tiny at the first few hours, thus, no observed change was detected. With increasing incubation time, these changes become visible. The inhibition of cell growth is a result of all mechanisms mention before.This proved that for specific concentration of silver nanoparticles, time is a critical factor in the inhibition process.

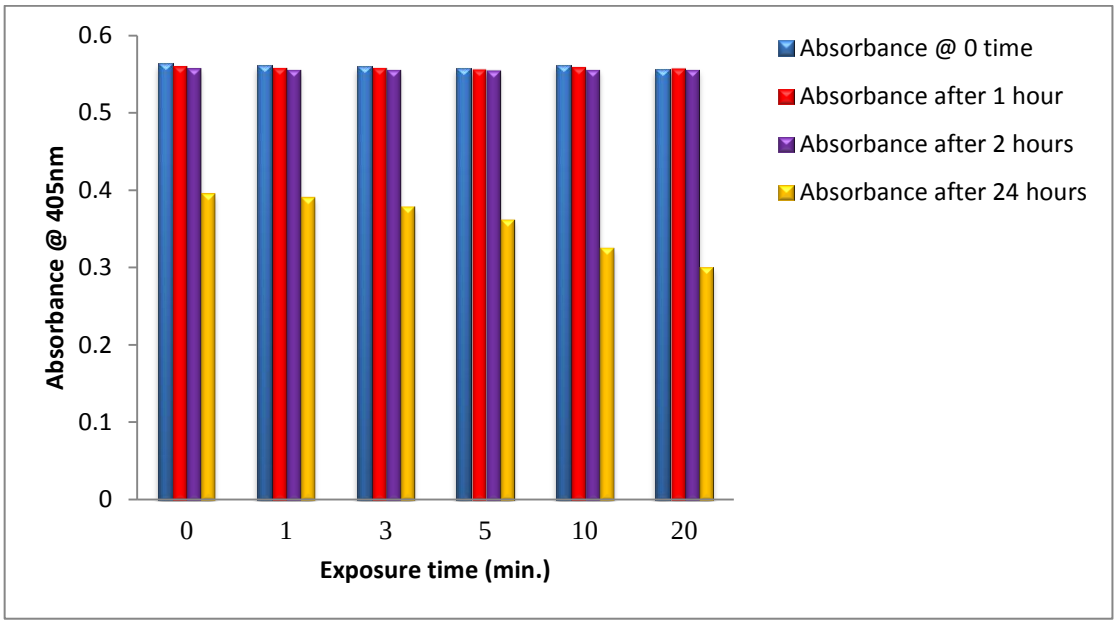


**Figure 6: Effect of 405 nm laser light (at deferent exposure times) and AgNPs (16 µg $ml^{-1}$) on the viability of *Pseudomonas aeruginosa***

It is possible that the mechanism of inhibition are affected by physiological state of the bacteria, such as the case of the phase of growth. When bacterial cells are introduced into fresh nutrient medium usually no

initial increase in cells number occurs, this period is called the lag phase, where the bacteria increase in size to start cell division. The second phase which is called exponential phase or logarithmic phase, here cell proliferation on its maximum. Third phase is the stationaryphase, some cells are dying while other still growing, this phase is known as death phase. Bacterial cells become more sensitive during log phase. This phase starts after certain time, thus, the cytotoxicity of AgNPs delayed for some time then appear obviously [8, 34-37].

Laser light which is not affect the bacterial growth by itself, intensify the toxicity of AgNPs via different mechanisms. Firstly; Light with a wavelength of 405 nm is capable of generating reactive species predominantly ROS in living cells after its absorption by endogenous photosensitizers such as porphyrins, cytochromes, and NADH[32,17,31]. The total quantities of ROS generated by laser light and AgNPs is high enough to start cell death. Secondly; laser light cause alteration of cell membranepermeability[35,36], this will in turn allow for more AgNPs particles to penetrate across the membrane and reach the intracellular components.

## Conclusion

This present research included the preparation of silver nanoparticles via laser ablation method and testing the antibacterial action of these nanoparticles with the bacterial cell alone or in combination with near UV laser light. Possible mechanism of this interaction is illustrated. Our results indicated good antibacterial action of synthesized AgNPs alone. The irradiation of bacterial suspension containing AgNPs with 405 nm laser light maximize the long term cytotoxicity of AgNPs detected after 24 hours following the treatment.

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