Comparative study on effects of antibacterial gold and silver nanoparticles on the survival of *Staphylococcus aureus* bacteria with laser light

Huda kadhem mohseen[1], Sadiq Hassan[2], Kaiser N. Madlum[3]

[1] University of Al-Qadisiyah/College of medicine ,[2]University of Babylon / college of Science for Women,[3] University of Babylon/College of medicine
Emails: huda.alsadiy.qu.edu.iq, sadiq.hassan74@yahoo.com, kaiser.madlum@gmail.com

Abstract

Nanomaterial science has emerged as a promissing field connecting different field togather. In this work, Gold and silver nanoparticles were prepared using a laser ablation technique. Nanoparticle solution was characterized using a UV-visible spectrophotometer, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Results revealed cytotoxic effects of Ag and Au NPs appear after long exposure time. These effects were enhanced by the irradiation of NPs after mixing with bacterial suspension. As a conclusion, Ag and Au NPs can be utilized as antibacterial agents under certain condition and with certain properties.

الخلاصة

انبثق علم المواد النانوية كمجال واعد يربط مختلف الاختصاصات بعضها ببعض. في هذا العمل تم تحضير دقائق الذهب والفضة النانوية باستخدام تقنية التفتيت الليزرية. تم تشخيص محلول المواد النانوية بواسطة مطياف الاشعة فوق البنفسجية ـالمرئية، المجهر الالكتروني الماسح، والمجهر الالكتروني النافذ. أظهرت النتائج تأثيرات سامة لدقائق الذهب والفضة النانوية بعد فترات التعريض الطويلة هذه التأثيرات كانت تزداد بتشعيع الدقائق النانوية بعد مزجها مع العالق البكتيري. كاستنتاج، فان دقائق الذهب والفضة النانوية يمكن استخدامها كمواد مضادة للبكتيريا تحت ضروف معينة وبخصائص محددة.

Introduction

Antibacterial agents are very important in many fields related to human life[1]. Nanoparticles (NP) are promising kind of antimicrobial agents , expected to open more new aspects to fight and prevent diseases[2]. The mechanisms of NP toxicity depend on its composition, surface modification, physical properties, and the bacterial type [3] .

Among all the nanomaterials that have antimicrobial properties, metallic NPs are the best [2]. These nanoparticles show unique physical and chemical properties arises specifically from their higher surface to volume ratio [4]. Silver and gold nanoparticles are widely used in different studies using numerous biological systems [3]. Silver and gold nanoparticles can simply be synthesized by top-down techniques (physical methods such as thermal decomposition, diffusion, irradiation) or bottom-up techniques (chemical polyol synthesis method, electrochemical synthesis, chemical reduction) [5][6]. Lasers reveal many new applications in the fields of microbiology, nanotechnology and nanobiotechnology. Recent studies concerned with the interaction of nanoparticles with laser light to enhance their antibacterial action[7].

Low level laser irradiation had an inhibitory effect on the many types of microorganisms. Laser light affects bacterial growth via different pathways mainly physiologically by the activation of singlet oxygen generation inside the cells[8][9][10].

Thus, it is worth to study the synergic effect of this physical agent with nanoparticles on the growth of the bacteria.

The growing resistance toward knowing antibacterial drugs has prompted a search for alternative antimicrobial agents.[11]. The resistance of bacteria to antibacterial agents has increased in recent years. Some antimicrobial agents are extremely toxic for the human. Nanoparticles interaction with biomolecules and microorganisms bring more attention to their antimicrobial action [4]. Interaction of the NPs with the bacterial surface can change the membrane properties. The small size with a very large surface area of nanoparticles allows them to adhere to the microorganism's surface, and then it can easily penetrate the cell and reach the inner contents[7].

Staphylococcus aureus bacteria responsible for numerous infections world- wide.[12]. It is commonly associated with wound infections besides being the most virulent species of its genus.[13].

The objective of this study was to compare the antibacterial effects of laser and gold and silver nanoparticles against *Staphylococcus aureus* bacteria in vitro.

Materials and methods

1- Synthesis and Characterization of gold and silver nanoparticles

Gold and Silver nanoparticles were prepared by Laser ablation method. Briefly, 2 g of highly purified (99.99 %) silver and gold plates (1x1 cm) were used as a target metal separately. Each piece was immersed into 2 ml of distilled water in the bottom of the quartz cell. Laser ablation was performed using a Q-switched Nd: YAG laser with a wavelength of 1064nm, a frequency of (5) Hz , pulse energy of (700) mJ and pulse width of (7) ns. The total number of pulses used were (50) pulses.

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).

2- Bacteria

Staphylococcus aureus isolated from patients with superficial wounds in Hilla surgical hospital. Characterization and identification of the bacterium were carried out depending on the morphological characteristics and biochemical tests. This gram-positive bacterium was used to evaluate the antibacterial effect of gold and silver nanoparticles. Briefly, Bacterial cells were suspended and diluted using the nutrient broth to 10^{-5} dilution which was equivalent to a bacterial concentration of approximately 10^6 cells/ml, confirmed via plate counts method. Bacterial suspensions were treated with different concentration of gold and silver nanoparticles.

3- Laser

Continuous wave semiconductor laser with a wavelength of (405 nm) and Nd:YVO4 laser (532 nm) with an output power of (10 mW) were used for irradiating the bacterial suspension.

4- Antibacterial activity measurments

1- Optical method

Antibacterial activity was studied using the optical method according to Amin et al, 2009 method with some modification [14]. Briefly, the bacterial suspension was incubated overnight in nutrient broth agar to activate the cells, then 1 ml inoculum mixed with 9 ml of sterile nutrient broth agar to prepare the stock. Serial dilutions were prepared from this stock, 200 µl from the 5th dilution was transferred to each sterile well of 96-well plate. Sterile flat bottom 96-well plates were used in this study. From the stock of nanoparticles, different volumes were added to each well until it reaches the required final concentration. The growth rate in the 96-well plate was monitored depending on the optical density (OD) with a microplate reader (Huma Reader HS, Japan). OD was measured at a wavelength of 405 nm due to high absorbance of bacterial suspension at UV region (figure 4). 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.

The effect of laser light on the viability of the bacteria in the presence of NPs was performed by irradiating bacterial samples containing (16 µg/ml) of NPs for 1, 3, 5, 10, and 20 minutes and the effect was monitored after 1, 2, and 24 hours.

2- Agar wells diffusion method

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

5- statistical analysis

The data were statistically analyzed using Sigmaplot version 12.0 software. Statistical significance was tested using a Student's t-test for unpaired observations. P values ≤0.05 were considered as statistically significant.

Results and Discussion

Characteristics of synthesized nanoparticles

Silver nanoparticles (Ag NPs) solution appears with a dark yellow color as shown in Figure 1 (right). UV-Visible spectrophotometric analysis of silver nanoparticles is shown in Figure 1 (left) where the peak absorbance at (420-425) nm. Figure 2 shows the AuNPs with its red wine color (right) and its UV-Visible spectrophotometric analysis (left) with peak absorbance at 540 nm.

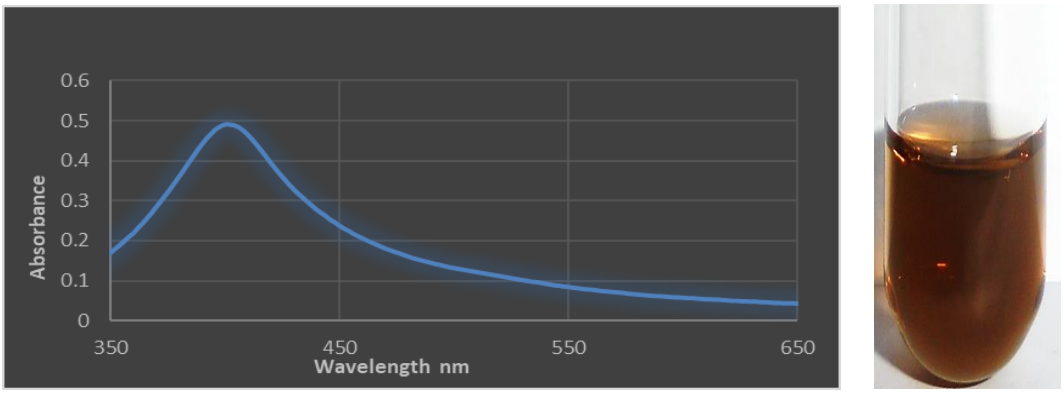

Figure 1: Silver nanoparticles. (left) UV-Visible spectra (right) the dark yellow color of Ag NPs solution

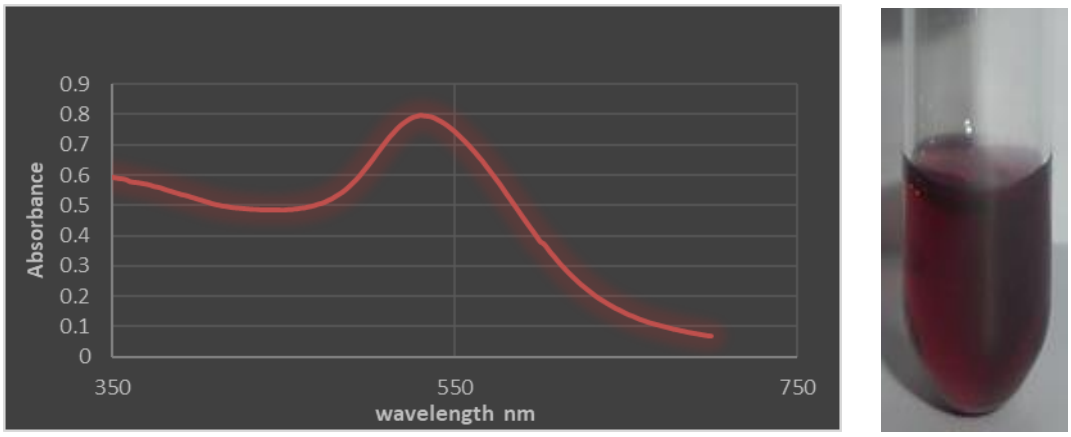

Figure 2: gold nanoparticles. (left) UV-Visible spectra (right) the red wine color of AuNPs solution

Data shown in figure 3 reveled the synthesis of Ag NPs in the ranges of (10-40 nm) with predominant of 20 nm particle size. Au NPs produce using this technique are much smaller than Ag NPs and show predominant particle size near 8 nm. These dimensions are close to previous dimensions obtained by laser ablation and chemical reduction method[6][15][16]

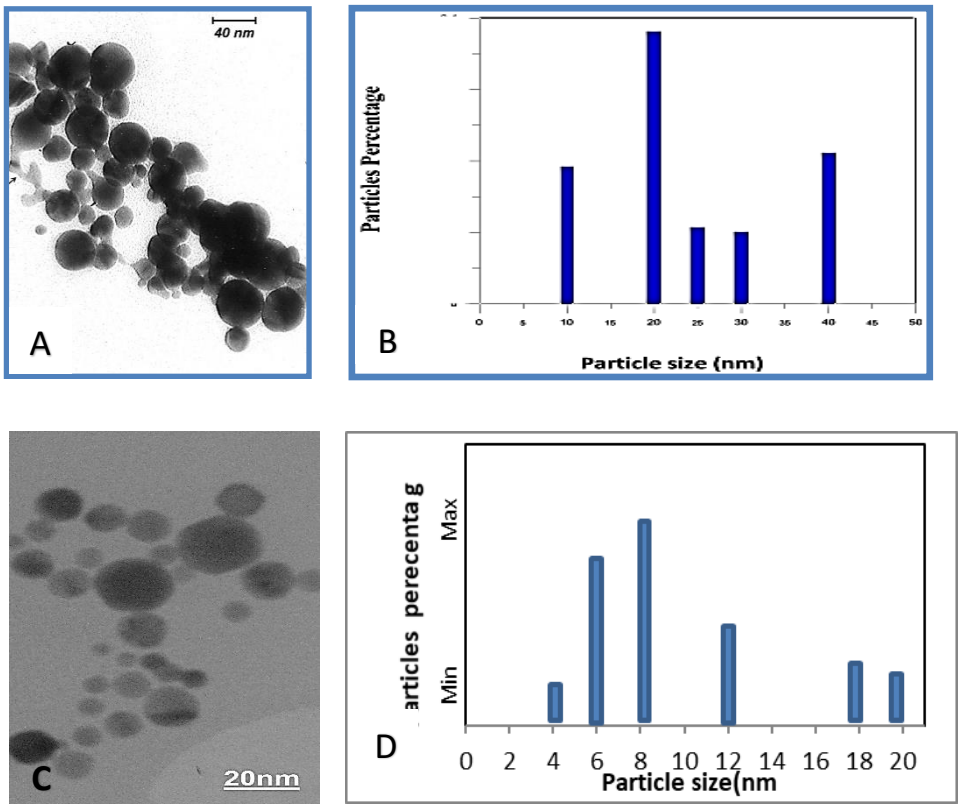

Figure 3: Nanoparticles dimensions and size distribution. (A) & (C) show TEM images, (B) & (D) show particle-size distribution of Au and Ag NPs respectively

Antibacterial activity of synthesized nanoparticles

In order to use the microbial optical density to monitor bacterial growth, the absorption spectrum of bacterial suspension was recorded at different wavelengths. As shown in figure 4, bacteria have a high absorption at the ultraviolet region also considerable absorption still observed in the visible region. Thus, the wavelength of 405 nm was used to record the absorption of the bacterial growth. Although Ag NPs have a relatively high absorption in this region, the initial measured OD is related to the bacterial plus Ag NPs absorption, but the change in OD will be restricted to the change in bacterial population, since Ag NPs are stable and its total numbers are not altered.

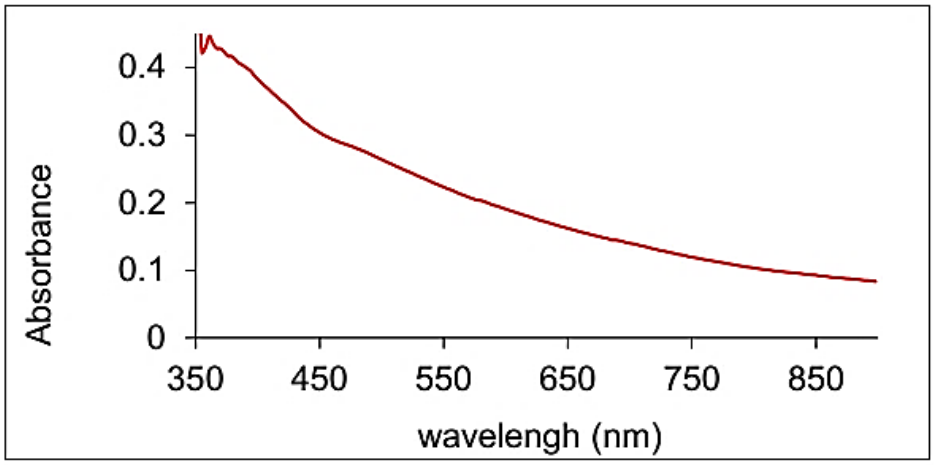

Figure 4: Absorption spectrum of the bacterial suspension

Figures 5 and 6 illustrate the effects of silver and gold nanoparticles on the growth of *Staph. aureus* bacteria. The concentration used ranged from 0.5 to 16 µg/ml for Ag NPS and from 2 to 32 µg/ml for Au NPs. obviously, there were nearly no effects for all concentrations used and after all treatment times applied except after 24 hours which showed a significant (P ≤0.05)decrease in bacterial numbers compared to the control

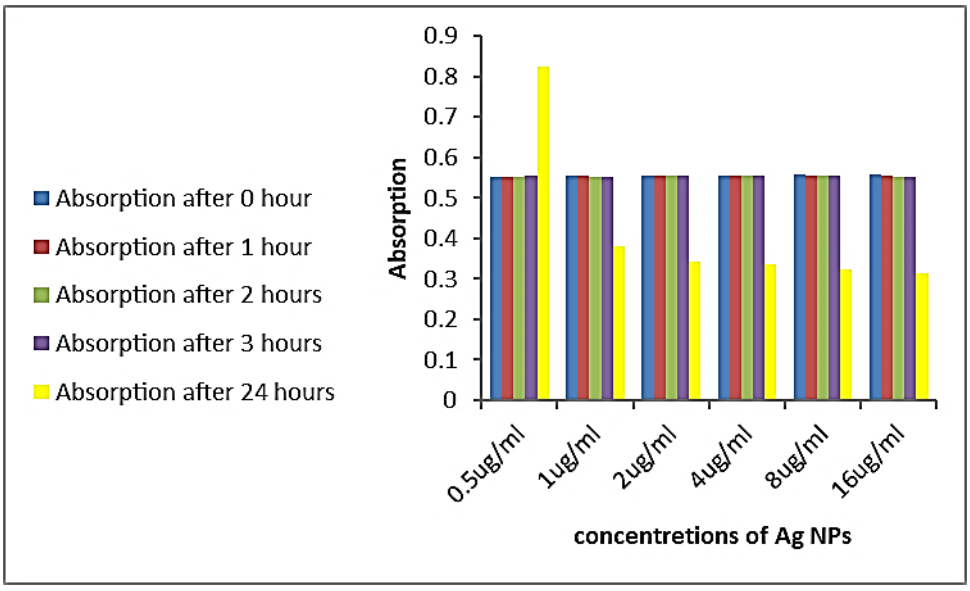

Figure 5: Antibacterial effect of AgNPs on *Staph. aureus* bacteria

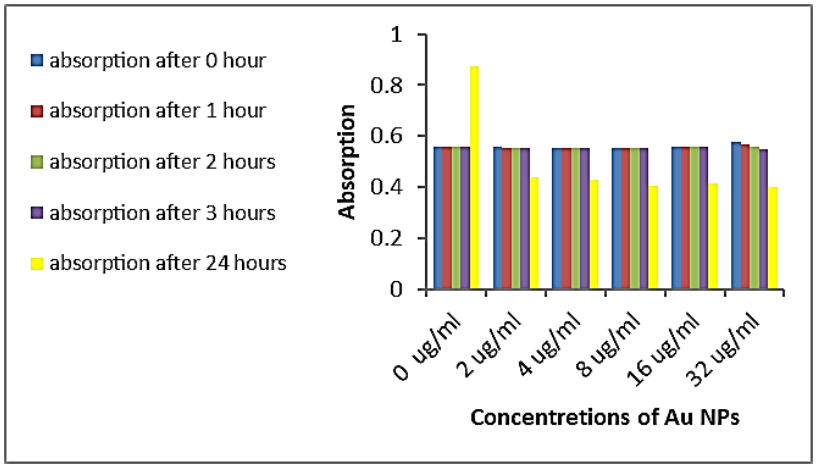

Figure 6: Antibacterial effect of Au NPs on *Staph. aureus* bacteria.

Figures 7 and 8 show the effects of laser lights (532 and 405 nm) on the growth of *Staph. aureus* bacteria. Both these wavelengths show no significant effects on these bacteria. The next step was to evaluate the effect of laser light on bacterial growth and survival in the presence of nanoparticles. Thus, the highest concentrations of NPs were used (16 and 32 µg/ml for Ag and Au NPs, respectively).

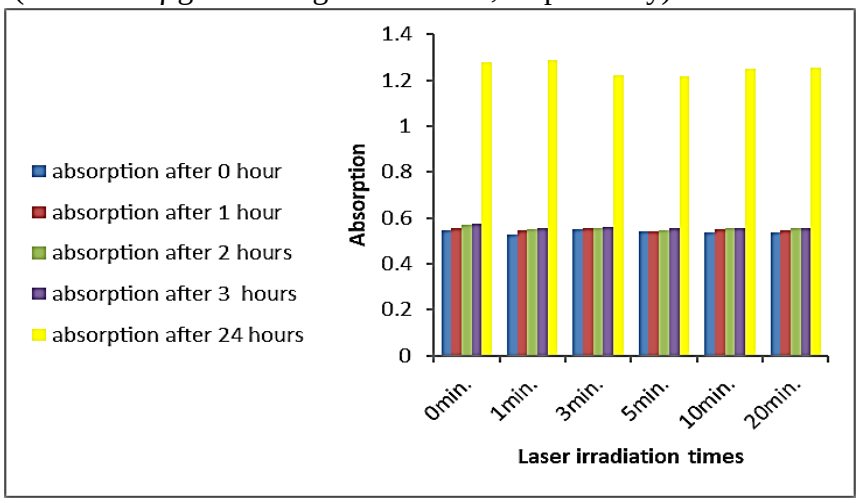

Figure 7: Effect of Nd:YVO4 laser (532 nm) light on *Staph. Aureus*

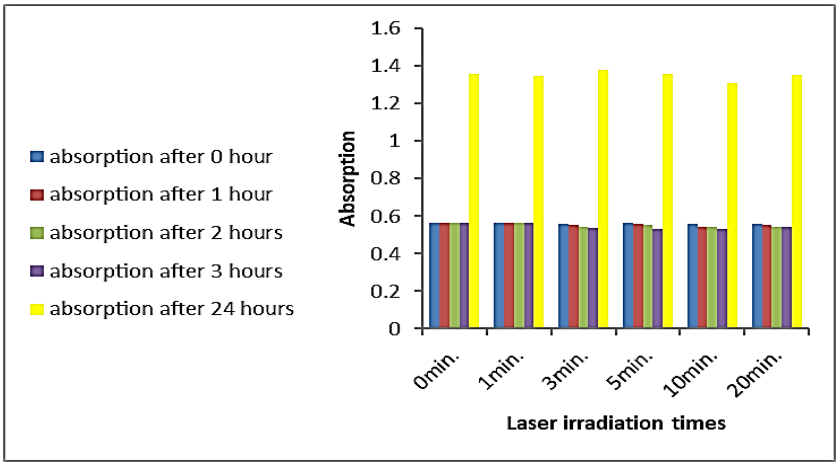

Figure 8: Effect of diode laser (405 nm) light on *Staph. aureus*

From figures 9 and 10, the effects of nanoparticles were clear only after long incubation times (24 hours) with both types of NPs. Laser lights enhance these cytotoxic effects especially after 24 hours and it was more effective in the case of gold nanoparticles.

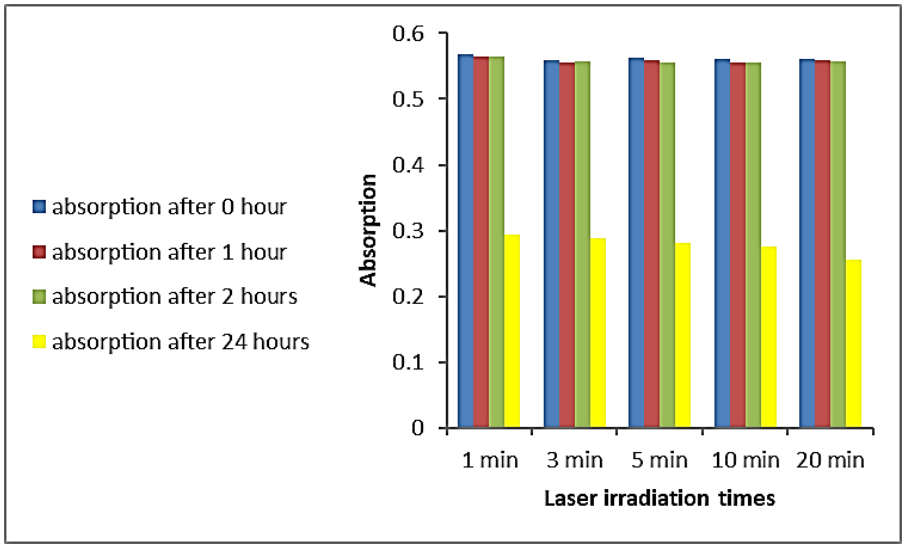

Figure 9: Effect of laser light (405nm) on the survival of *staph. aureus* bacteria in the presence of (16 µg/ml) of Ag NPs

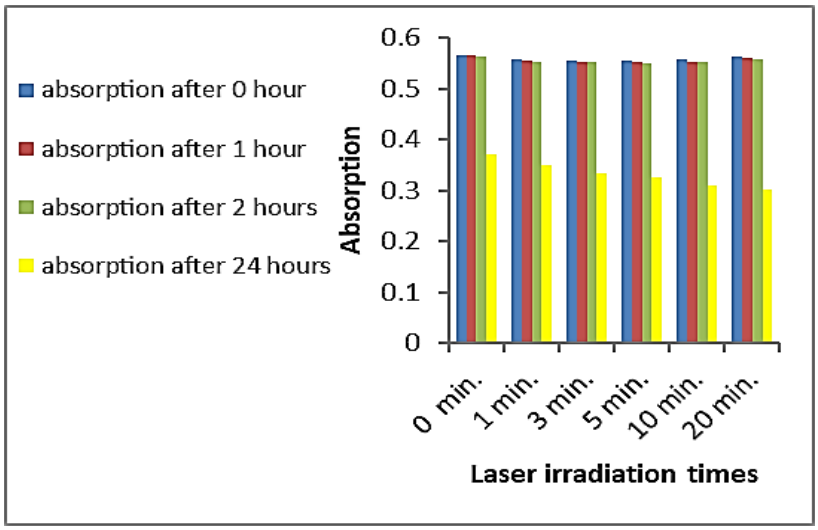

Figure 10: Effect of laser light (532nm) on the survival of *staph. aureus* bacteria in the presence of (32 µg/ml) of Au NPs

Table 1: size of the inhibition zone produced by the nanoparticles

Ag NPs concentrations (µg/ml)	Inhibition zone (mm)	Au NPs concentrations (µg/ml)	Inhibition zone (mm)
0.5	8.8	1	0
1	9	2	6
2	11	4	9
4	13	8	20
8	18	16	22.5
16	22	32	28

Discussion

Interaction of nanoparticles with microorganisms is expanding the field of research. The antibacterial activities of Ag compounds are well knowing. Previous studies have shown that Ag nanoparticles can be used as effective and safe bactericidal agents[4]. Photoactive nanoparticles have shown different extents of cytotoxic effects. The most important factor related to its activity is the generation of Reactive oxygen species (ROS) that can damage DNA and RNA strands in cells leading to apoptosis processes. It was found that Ag NPs increased the concentration of ROS, and thus caused cell death. Many factors affect NPs cytotoxicity, mainly size, shape, and concentration. Gold nanoparticles (Au NPs) have less cytotoxic effects due to the nature of inert elements. The effect of size is different than that of Ag NPs, cytotoxicity increases with decreasing the size [17][18]. In our work, Ag NPs were about 20 nm size. This size is not small enough to cause sever toxic effects. The penetrating ability of this small size particles into cells have made AuNPs excellent carriers in biomedical and molecular biology techniques. The feature has eased the processes of penetration, absorption, circulation and distribution of AuNPs in the host's cells without cell injury and toxicity[19].

Cell types also affected the cytotoxicity of Au and Ag NPs. The Ag NPs affect Gram-negative bacteria more than Gram-positive bacteria. The possible explanation for this difference is due to the difference in cell wall structure that protects the cell against the penetration of NPs. Moreover, both antibiotic resistance and susceptible bacteria show the same response to Ag NPs [20] [18].

Furthermore, the influence of Au and Ag NP toxicity has also been shown to vary with particle shapes. It was found that rods shaped AuNPs have been reported to cause more toxicity than spherical particles [19]. As for most chemicals, NPs effects increases with increasing their concentrations [12]. Thus, cytotoxicity of our NPs was shown to be more marked at higher concentrations.

The clarity of toxicity needs for a time. Thus, it appears only after 24 hours of treatment. This may due to the fact that the appearance of the effect strongly depends on the difference in bacterial number between the control and the treatment groups.

Laser lights used in this study has an indirect effect on bacteria via activating the nanoparticles instead of direct effects on cells since its power was too low [21][22]. Authors used an infra-red laser to activate gold and silver nanoparticles found that treatment of bacteria with Ag NPs plus laser but not Au NPs caused significant cytotoxic effects [22]

The method used in this study (optical method) using broth microdilution is a suitable and fast screening method for MIC determination compared to other methods.[20].Similar results obtained by agar well diffusion method (table 1). Here, the toxic effects appear more lucidly than that in the optical method. Moreover, the toxic effect of Ag NPs started to appear earlier than that of Au NPs. The concentration of 0.5 µg/ml of Ag NPs creates inhibition zone larger than that created by 2µg/ml of Au NPs. Many authors consider this method as the most accurate, sensitive, and rapid tool to examine the antibacterial efficiency [23][20].

Conclusions

Gold and silver nanoparticles have the ability to overcome the antibiotic resistance of some bacteria. These nanoparticles target bacterial cells in various mechanisms. The extent and severity of cytotoxicity depend on many factors namely particle size, shape, exposure time and concentration. Laser lights accompanying NPs can result in butter antibacterial activity.

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