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Research paper

Antimicrbial Effect of Diode Laser and Biossynthesis Silver Nanoparticles on Bactrium Staphylococcus Aureus in Vitro

Raad Sh. Alnayli[1], Adnan H. Al Hamadani[2],Safaa A. Yaseen[3]

[1]*Department of Physics /College of Education /University of Al-Qadisiyah,Iraq. Email: Raad. Alnayli@qu.edu.iq.*
[2] *Department of Microbiology/ College of Medicine/ University of Al-Qadisiyah,Iraq. Email: adnan.uobeed@qu.edu.iq.*
[3]*Department of Physics /College of Education /University of Al-Qadisiyah,Iraq. Email: safaaameer292@gmail.com*

Abstract

The present work was aimed to illustrate how the use of Diode laser or silver nanoparticles (AgNPs) each of them individually, as an antibacterial agent against *Staphylococcus aureus* (*S. aureus*) as well as study the effective result from the absorption of laser energy by these nanoparticles to kill or inhibition bacterial growth. The silver nanoparticles are prepared by biological method. *S. aureus* are isolated and identified in the Central Health Laboratory in Al Najaf city. Nanoparticles are tested against *S. aureus* cultured on Muller Hinton agar but Diode laser or laser with nanoparticles are tested against *S. aureus* cultured in Nutrient broth. *S. aureus* are irradiating by Diode laser with different irradiation times, moreover there are different concentrations of AgNPs have been employed for killing and inhibition bacterial growth. Results showed that *S. aureus* is affected by AgNPs (mainly highest concentration of nanoparticles) also detected that decrease bacterial availability with increase time of irradiation to laser.

Key words: *Diode laser, AgNPs, Nanoparticles, S. aureus*

1.Introduction

Drug resistance by pathogenic bacteria such as *S. aureus* remains worldwide problem [1]. Therefore, we require finding out novel strategies or recognizing new antimicrobial agents to control microbial infections. Superior effectiveness on resistant strains of microbial pathogens, less toxicity and heat resistance are the characteristic of laser and metal oxide nanoparticles, which make them the selective candidates for eradicating bacteria [2,3]. The small size of silver nanoparticles referred which is 250 times smaller than a bacterium that might be giving it the antimicrobial ability [4]. The mechanism of bactericidal actions of silver nanoparticles is still not well understood. In a previous report on the bactericidal activity of silver nanoparticles, it was shown that the interaction between silver nanoparticles and constituents of the bacterial membrane caused structural changes in and damage to membranes, finally leading to cell death [5,6]. Microbes are unlikely to develop resistance against silver, as they do against conventional and narrow target antibiotics, because the metal attacks a broad range of targets in the organisms, which means that they would have to develop a host of mutations simultaneously to protect themselves [7-10]. The laser used in medical field for different purposes like to cure various types of ulcerous wounds. The increase in the exposure of the radiation resulted in the reduction of the bacteria count due to their destruction because of the radiation [7-12]. Finally there are a little attention has been given to the bactericidal effect by Diode laser radiation on *S. aureus*.

The aim of the study:

The present study was aimed to identify the antibacterial effect of Diode laser and silver nanoparticles (alone or in combination with each other) on one of pathogenic bacteria (*S. aureus*).

2.Materials and Methods

2.1.Preparation of Nanoparticles

Silver nanoparticles are prepared by the biosynthesis method [13] in the Microbiology Laboratory- for postgraduates research - College of medicine - University of Al-Qadisiyah.

2.2.Biosynthesis method of AgNPs:

***I-Preparation of Fungal Culture*:** *Aspergillus niger* was grown in yeast malt broth at 37 °C for 5 days. The flasks were incubated in the shaker incubator at 200 RPM (Revolution per minute). After 5 days of incubation, the mycelium was separated and washed thrice with deionized water. 20 g of biomass was treated with 200 ml of deionized water for 72 h at 250 °C in an Erlenmeyer flask and agitated in the same condition as described earlier. After the incubation, the cell filtrate was obtained by filtration through Watmann filter paper number 113.

II- Synthesis of Silver Nanoparticles: Silver nitrate at 1mM concentration was mixed with 50 ml of cell filtrate (above) in a 250 ml Erlenmeyer flask and agitated at 25 ºC in dark along with control 13. Colloidal nano silver that has particle size 20 nm to 40 nm (average = 30 nm) (nm: mean nanometer) with different concentration (36.21 ppm) that equal to mg/L (This concentration was diluted to one half and quarter).

2.3.Preparation of Bacterial Samples

The bacterial sample was *S. aureus*, collected from the surgical tools in the operation rooms of the Al- Najaf teaching hospital then *S. aureus* isolated and identified in the Central Health Laboratory from Al Najaf city.

2.4.Antibacterial effect of nanoparticles

To examine the susceptibility of *S. aureus* to different silver nanoparticles, *Muller Hinton agar* were prepared with holes, the diameter for each hole was 5 mm. Placed 0.2 ml of each concentration of AgNPs in one hole. Put the plates in the incubator for 24 hrs in temperature of 37 °C then zone of inhibition measured manually.

2.5.Application Laser Irradiations on *S. aureus*:

The procedure for applying the Diode laser (with 50 mW) on the bacterial samples includes take tubes that contain *S. aureus* cultured in *Nutrient broth* and irradiate them with Diode laser with different exposure times (1,3,5,7,10,15 and 20 min).

2.6.Application Laser Irradiations with Nanoparticles on Bacterial Species:

The procedure for applying the Diode laser (with 50 mW) on the bacterial samples includes take tubes that contain *S. aureus* bacteria cultured in *Nutrient broth* with different concentrations of AgNPs and irradiate them with Diode laser with different irradiation times (1,3,5,7 and 10 min).

3.Results

3.1.Nanoparticles test

The size and morphology of nanoparticles were determined by UV-Vis spectroscopy in College of medicine - University of Al-Qadisiyah and scanning electron microscope (SEM) in College of Science, University of Al-Kufa as seen in fig.1 and fig. 2.

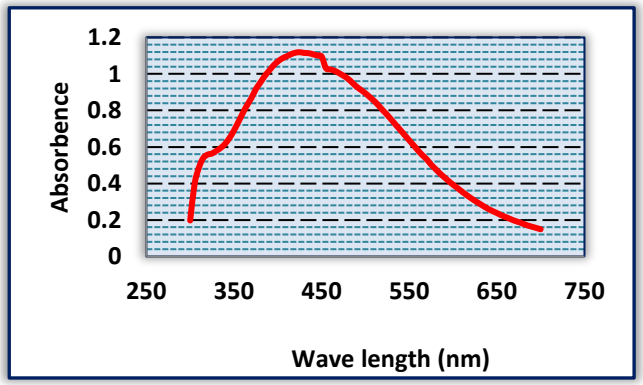

Fig. 1: Represent absorption spectrum for biosynthesis of AgNPs.

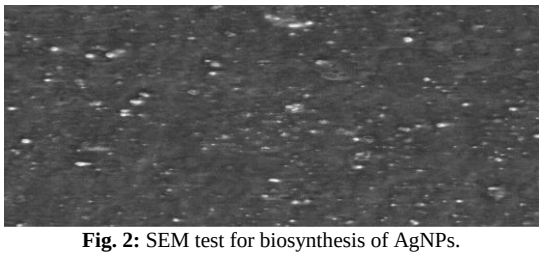

Fig. 2: SEM test for biosynthesis of AgNPs.

3.2.Antimicrobial Effect of Nanoparticles on *S. aureus*

The effect of AgNPs on *S. aureus* can be seen in fig.3. Results showed that sensitivity of *S. aureus* to nanoparticles significantly associated ($p<0.05$) with increase concentration of nanoparticles (fig. 4).

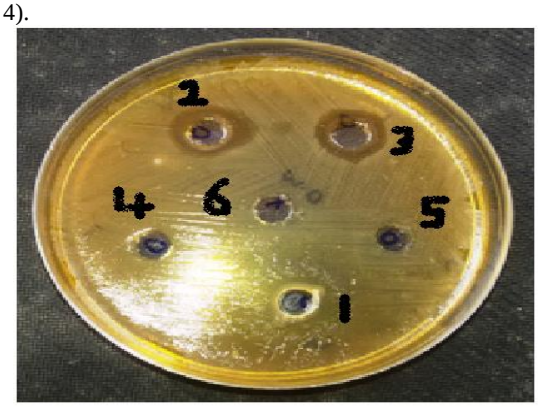

Fig.3: Represent image for effect of AgNPs on *S. aureus* that cultured on Muller Hinton agar. Where: 1, 2, 3 represent concentrations of biological AgNPs with 36.21 ppm, 18.1 ppm and 9 ppm respectively.

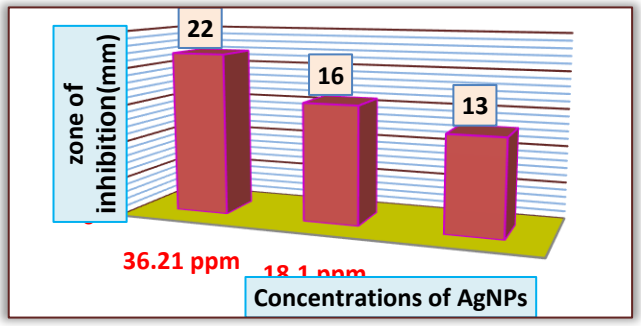

Fig. 4: Effect of AgNPs (zone of inhibition) in different concentrations on *S. aureus* ($p<0.05$), ppm: part per million.

3.3.Effect of Laser Irradiations on tested Bacterial Species

The effect of Diode laser (50 mW) in different irradiation times on *S. aureus* can be seen in the fig. 5 that showed increase irradiations times significantly associated ($p<0.05$) with decrease bacterial availability in *Nutrient broth* (low absorbency).

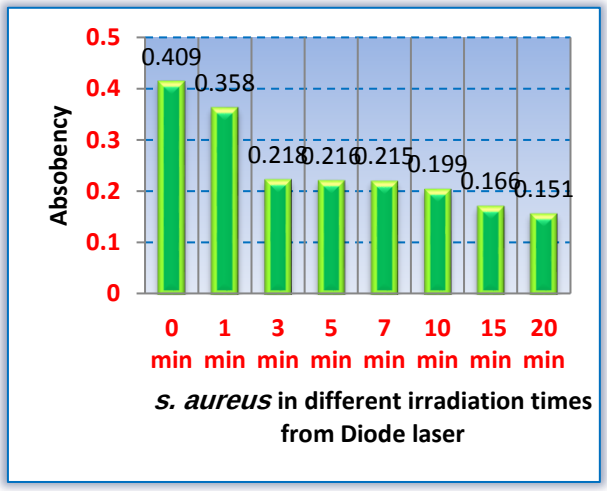

Fig. 5: Represent Effect of Diode laser in different irradiation times ($p<0.05$).

3.4.Mutual Effect of Laser Irradiation and Nanoparticles on Bacterial Species

Fig. 6 showed the effect of Diode laser (50 mW) with AgNPs on *S. aureus*. Although the effect of Bacteria by dual action of Laser Irradiation with Nanoparticles are very little, or non-significant ($p>0.05$) the inhibitory effect mainly appeared at long Irradiation time (10 min) with highest concentration of nanoparticles.

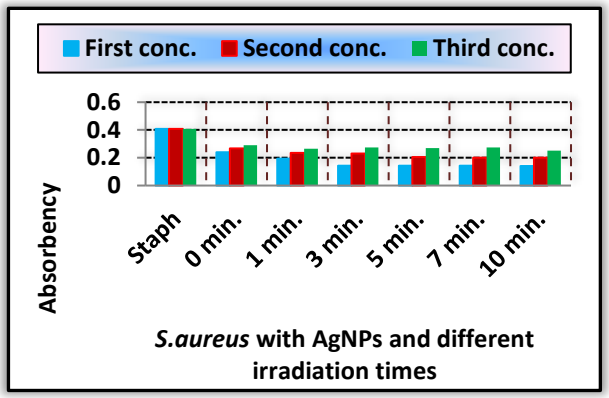

Fig.6: Represent Effect of Diode laser in different irradiation times with AgNPs in different concentrations on *S. aureus* ($p>0.05$).

4.Discussion

This study reported that Silver nanoparticles have inhibitory and bactericidal effects against *S. aureus* and this agree with study of Guzman *et al* (2012) who show that AgNPs have bactericidal activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *S. aureus* [14-16]. Al-Nori (2012) showed that Silver nanoparticles destabilize plasma membrane and depletion of levels of intracellular adenosine triphosphate (ATP) by targeting bacterial membrane resulting in bacterial cell death [17-19]. In this study antimicrobial action of used nanoparticles is enhanced with increase their concentration and this agree with other studies [2,14]. Current study detected inhibitory action of diode laser on *S. aureus* and this agree with study of Ismail *et al* (2012) who show that diode laser light reduce *S. aureus* growth but not effect on the enzymes production (Catalase and Coagulase), and the fermentation of Mannitol of these isolate [20]. Present research determine bacterial inhibition increase with increase time of exposure to diode laser radiation and this in consent with other studies [15-22]. In the current study, the dual action of laser and nanoparticles not have clear inhibitory action on *S. aureus* and this agree with study of Hassan, (2015) who determine that tested *S. aureus* can re-grow after 24 hr of incubation [23].

5.Conclusion

1- The inhibited *S. aureus* was increased significantly with the concentration of the silver NPs.
2- Increase time of irradiation by 650 nm laser cause significant increase in the killing of bacteria especially after10 min of irradiation.
3- The effect was increasing when NPs utilizing by using the same period.

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