Antimicrobial Effect of Silver Nanoparticles and He-Ne Laser on Bacterium *Staphylococcus Aureus* in vitro

Assist prof. Dr. Khalid I. Riah

*Department of Physiology and Medical Physics, College of Medicine, University of Al-Qadisiyah, Iraq. Email: Khalidalfatlee@gmail.com and Khalid.Ibrahim@qu.edu.iq.*

**Abstract**

This study shows the effect of silver nanoparticles (Ag-NPs) or He-Ne laser (2mw) each of them individually, on *Staphylococcus Aureus* (*S. aureus*); also study the dual effect of nanoparticles and laser energy to kill or inhibition of bacterium. He-Ne laser with different time of irradiation and Ag-NPs with different concentrations were used against *S. aureus* bacterium. The results showed that *S. aureus* was affected by Ag-NPs especially in high concentration and there was a little effect of He-Ne laser on bacterial growth.

**Key words: laser, He-Ne, nanoparticles, Ag-NPs, S. aureus.**

1. **Introduction**

The field of nanotechnology is one of the most active areas of research in modern material science. Silver has long been recognized as one of the nanoparticles having inhibition effect on microbes found in medical field [1]. Among the noble metals, silver (Ag) is the metal of choice in the field of biological system and medicine [2]. Also silver nanoparticles (Ag-NPs) have received its important because of owing to their attractive physicochemical properties [3].
Ag-NPs are a nontoxic, safe inorganic antibacterial to a long range of micro-organisms; so silver-based compounds have been used extensively in wide antibacterial applications [1]. Wide range of the bacteria has yet developed resistance to antibiotics which in future it is need to develop a substitute for antibiotics. Ag nano-particles are attractive, nontoxic to human body at low concentration and having wide-spectrum antimicrobial nature [3].

The antimicrobial effect of Ag-ions on micro-organisms is very well known; however, the bactericidal mechanism is only partially understood. Practical evidence suggests that DNA loses its replication ability once the bacteria have been treated with Ag-ions; other studies have shown evidence of structural cell membrane changes [4].

We chose Staphylococcus aureus (S. aureus) bacterium, as it is commonly associated with injury infections and it is the most common gram positive bacterium in infections of wounds, in addition for being the most virulent species of its genus [5].

Low level lasers intensity (LLLI) has been used for many years for the treatment of a wide range of medical conditions including soft tissue injuries and wound healing [6]. The biological effects of LLLI are dependent on the exposure parameters used. Directionality, high monochromaticity, energy densities, and emission mode properties are characteristics that make laser devices to treat various diseases [7].

The best known and most widely used He-Ne laser operates at a wavelength of 632.8 nm with red colour. It is highly compact, reliable. The mechanism sequence for killing of bacteria has been reported to involve the formation of singlet oxygen and free radicals [8-10].

**Objective of the study:**

The coal of this study was to illustrate the bactericidal effect of different concentrations of silver nanoparticles and He-Ne laser with two irradiation time (alone or together) on pathogenic bacterium (*S. aureus*).
2. Materials and Methods

2.1. Bacterium

*S. aureus* bacteria sample were obtained from the appliances in the surgical rooms of Al-Sadder hospital in Al-Najaf city, Iraq, after that, they were tested in the Al Najaf Central Laboratory for Health. The collection was done by swab and then *S. aureus* were isolated and identified by use MacConkey agar and biochemical tests.

2.2. Preparation of Nanoparticles

The preparation was done in the Lab of Nano-technology - Engineering College of - Al-Kufa University of, Iraq.

The preparation of Ag-NPs was prepared using laser ablation technique, by Nd-YAG laser with wave length 1064 nm and 1000 mj maximum energy for each pulse. Colloidal Ag-NPs result with (20 - 40 nm) particle size and with concentration 250 ppm (ppm= part per million), ppm equal to mg/L in unit.

The 250 ppm was diluted to get other concentrations (50 and 15 ppm) that prepared according to the dilution equation (11):

\[ C_1V_1=C_2V_2 \]

Where:

C1 and C2: represent the original and final concentrations. V1 and V2 represent the volume that need to dilute it and the wanted volume that result from the dilution alternatively.

2.3 Helium Neon laser (2 mw)

He-Ne laser was used with 632.8 nm wave length and 2 mw power has a red colour, in the Lab of Medical physics, Medical college, Al-Qadisiyah University, Iraq.
2.4. Anti-bacterial effect of nanoparticles

The steps that followed for applying Ag-NPs on the bacterial species include takes tubes which contain \textit{S. aureus} cultured in Nutrient broth media and added 0.2 ml of colloidal Ag-NPs to them with the concentrations prepared previously. Then take 0.2 ml from each tube for later examination.

2.5. Application of He-Ne laser irradiations

This done by applying He-Ne laser on tubes that contains \textit{S. aureus} cultured in Nutrient broth media with two irradiation times (5 and 10) minutes.

2.6. Application of He-Ne laser irradiations with Ag-NPs

This part was done by using tubes that contain \textit{S. aureus} bacterium that cultured in Nutrient- broth media with Ag-NPs in various concentrations (15, 50 and 250) ppm and then apply He-Ne laser with the same irradiation times mentioned previously (5 and 10) min.

2.7. Elassa test

This part done by taking 0.2 ml from each tube that contain: 1- \textit{S. aureus} bacterium that exposed to Ag-NPs only.

2- \textit{S. aureus} bacterium irradiated with He-Ne laser only.

3- \textit{S. aureus} bacterium that exposed to Ag-NPs and irradiated with laser.

Put them in Tissue Culture Plate (TCP) and test them by determining the absorbency for the bacterium.

3. The results:

3.1. Nanoparticle test

The morphology and size of Ag-NPs testes were done by SEM (scanning electron microscope) in the Science College , Al-Kufa University and UV- Visible spectroscopy in Lab of Biology, Medical college, Al-Qadisiyah University that shown in figure (1) and figure (2).
3.2. Effect of Ag-NPs concentrations on tested bacteria

Increasing the concentration of Ag-NPs lead to increase the inhibition of S. aureus in all concentrations used significantly (p < 0.05) as shown in figure (3).

3.3. He-Ne laser irradiation Effect on tested bacteria

Irradiation by He-Ne laser with 5 min. has a little effect on S. aureus and this effect was increased when using 10 min. irradiation time. The two irradiation have no significant effect on S. aureus as seen in figure (4).
Figure (3): Effects of different Ag-NPs concentrations on S. aureus.

Figure (4): He-Ne laser irradiation effect on S. aureus with different times.
3.4 Combination effect of He-Ne laser irradiation and Ag-NPs concentrations on *S. aureus* bacteria

The combination effect of Ag-NPs with different concentrations used and He-Ne laser with 5 min. irradiation time cause inhibition in *S. aureus* significantly with increasing the Ag-NPs concentrations (Fig.5). The same behavior above was shown when using more irradiation time (10 min.) as seen in figure (6).

There was no significant difference between the two irradiation times used in killing the *S. aureus* when comparing them with the concentration used as shown in figure (7).

![Graph showing absorbancy against AgNPs concentrations with 5 min. irradiation time](image)

Figure (5): He-Ne laser irradiation (5 min.) with Ag-NPs effect on *S. aureus*. 
3.5. Statistical Analysis
Excel program form Microsoft Office software and SPSS Software were used to compare and draw the data gained during study.
4. Discussion

Iraq hospitals like many other countries hospitals sever from bacteria drug resistance. There has been an increasing in hospital and medical centers - acquired infections worldwide. In the Arab countries (including the Kingdom of Saudi Arabia) the prevalence of patients of multidrug resistant bacteria is on a rise [12, 13].

So, searching about exchangeable noble way to replace the common anti- bacterial materials with modern materials like nanoparticles was very important in present time.

There was direct relationship between the increasing the concentration of nanoparticles and inhibition in *S. aureus* bacterium (Fig. 3) especially in concentration of 250 ppm. This has acceptance with Mahmood study that reported Ag-NPs accumulated in the cell bacterial membrane caused change in permeability and causing cell death [14]. Also study of Sadeghi, et al., show that Ag-NPs exhibit more activity against tested positive and negative gram bacteria than shown in this study [15]. This may related to the low concentrations used in this study.

There was a little effect of He-Ne laser with first 5 min. of irradiation on *S. aureus* bacterium (Fig. 4) and this effect was increased in 10 min. of irradiation but does not reaching the required bacterial inhibition.

When comparing this results with other studies, we can observe that increasing the power of He-Ne laser to 5 mw (in another study) do not give great change relative to our result [16], but increasing the power to 50 mw with increasing the time of irradiation give more better result when using Diode laser [17,18].

Using Ag-NPs and He-Ne laser together against *S. aureus* bacterium (Fig. 5 and Fig. 6) does not enhance the result gained when using the Ag-NPs alone as shown previously (Fig. 3).
Also, there was no statically recognize difference between the two irradiation time used (5 and 10) min. as shown in figure (7). This has acceptance with another researcher use He-Ne laser with 5 mw with Ag-NPs [16].

5. Conclusion

In this study, the dominant effect was coming from Ag-NPs to inhibit the *S. aureus* bacterium when used alone or in combination with He-Ne laser. The minimal effect of He-Ne laser used may related to the low power (2 mw) and this type of laser may need more time to give accepted result for bacterial inhibition.

6. References

[1]. Akhilesh K, Vishal K, Juhi B, Priya S and Khadeeja Y. Isolation and identification of *E. coli* bacteria for the synthesis of silver nanoparticles: Characterization of the particles and study of antibacterial activity. European Journal of Experimental Biology, 2015, 5(1):65-70.

[2]. Matsumura Y, Yoshikata K, Kunisaki S, Tsuchido T. Mode of Bactericidal Action of Silver Zeolite and Its Comparison with That of Silver Nitrate. *Appl Environ Microbiol*, 2003 Jul; 69(7): 4278–4281.

[3]. Theivasanthi T, Alagar M. Anti-bacterial Studies of Silver Nano- particles. Department of Physics, PACR Polytechnic College, India 2010:1-5.

[4]. Jose R, Jose L, Alejandra C, Katherine H, Juan B, Jose T and Miguel J. The bactericidal effect of silver nanoparticles Nanotechnology 16 (2005) 2346–2353.

[5]. Pmela R; Josue B; Juliette C; Marcelo P; Luiz C and Tcbcp R. Effects of low intensity laser in in vitro bacterial culture and in vivo infected wounds. Rev. Col. Bras. Cir. 2014; 41(1): 049-055.

[6]. Wendy Ch., Jerrold S., Michael L, Jason L, Joon P, Judy L, Haneul L. The effects of low level laser radiation on bacterial growth. Phys Ther Rehabil Sci, 2014, 3 (1), 20-26. [www.jptrs.org](http://www.jptrs.org).

[7]. Barboza L, Campos V., Magalhães L, Paoli F and Fonseca A. Low- intensity red and infrared laser effects at high fluences on Escherichia coli cultures. Brazilian Journal of Medical and Biological Research (2015) 48(10): 945–952.

[8]. Satturwar M, Jagtap Sh, Rathod K. Effects of He-Ne laser irradiation on Escherichia Coli and Bacillus subtilis. International Journal of Basic and Applied Vol. 02 (2012) 13-19.

[9]. Millson C, Wilson M, Macrobert A, bedwell j. “The killing of Helicobacter pylori by low-power laser light in the presence of a photosensitise” (1996) Journal of Medical Microbiology, 44:245-252.
[10]. Cory C, Noelle A. Simone. “Bactericidal Effect of 0.95- mW Helium-Neon and 5-mW Indium-Gallium-Aluminum-Phosphate Laser Irradiation at Exposure Times of 30, 60, and 120 Seconds on Photosensitized Staphylococcus aureus and Pseudomonas aeruginosa In Vitro”(1999) Physical Therapy Vol.79: 839-846.

[11]. Kaiser, M. A. Utilizing of nanoparticles and laser energy for killing and inhibition on some pathogenic bacteria. M.Sc. Thesis, University of Babylon, College of Science for women, Dept. of laser Physics. Iraq. 2016, 17-18.

[12]. Vijayakumar, R.; Sandle, T.; Al-Aboody, M.S.; AlFonaisan, M.K.; Alturaiki, W.; Mickymaray, S.; Premanathan, M.; Alsagaby, S.A. Distribution of biocide resistant genes and biocides susceptibility in multidrug-resistant Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii. J. Infect. Public Health 2018, 11, 812–816, doi:10.1016/j.jiph.2018.05.011.

[13]. Balkhy, H.H.; Assiri, A.M.; Al, H.; Al-abri, S.S. Al-katheeri, H., Alansari, H.; Abdulrazzaq, N.M.; Aidara- Kane, A.; Pittet, D.; Erlacher- Vindel, E.; et al. The strategic plan for combating antimicrobial resistance in Gulf Cooperation Council States. J. Infect. Public Health 2016, 9, 375–385.

[14]. Mahmood, M. A. The antibacterial effect of silver nanoparticles on some bacterial pathogens. Iraqi Journal of Physics 2012, 10(18): 56-61.

[15]. Sadeghi B.; Jamali M.; Kia S.; Amininia A.; and Ghafari S., Synthesis and characterization of silver nanoparticles for antibacterial activity. Int. J. Nano. Dim (2010), 1(2):119-124.

[16]. Safaa A. Yaseen. Investigation of the effect of laser and nanoparticles on different species of bacteria. M.Sc. thesis, University of Al-Qadisiyah, College of Education, Dept. of Physics. Iraq. 2017, 56-57.

[17]. Raad Sh. Alnayli, Adnan H. Al Hamadani, and Safaa A. Yaseen. Silver Nanoparticles on Biosynthesis Effect of Diode Laser and Antimicrobial Escherichia Coli in Vitro. AL-Qadisiya Medical Journal, 2017, Vol.13 No.24:87-93.

[18]. Raad Sh. Alnayli, Adnan H. Al Hamadani, and Safaa A. Yaseen. Antimicrobial Effect of Diode Laser and Biosynthesis Silver Nanoparticles on Bactrium Staphylococcus Aureus in Vitro. International Journal of Engineering & Technology, 2018, 7 (4.36) 290-292.