Bactericidal effect of silver nanoparticles and He-Ne laser on bacterium *Escherichia coli* in vitro

Khalid I. Riah[1]

[1]Department of Physiology and Medical Physics, College of Medicine, University of Al-Qadisiyah, Iraq.

*Corresponding author: Khalid.Ibrahim@qu.edu.iq (Riah)

Abstract
This study was designed to show the effect of silver nanoparticles (Ag-NPs) or He-Ne laser each of them individually, on *Escherichia coli*, also study the combined effect of nanoparticles and laser energy to kill or reduction of bacterial growth. Both, He-Ne laser and Ag-NPs were used against E. coli. Various concentrations of Ag-NPs have been employed, as far as; different irradiation times of laser were used for reducing bacterial growth. The results showed that E. coli was affected by laser and Ag-NPs when used separately and that effect was increased when using them together.

Key words: laser, He-Ne, nanoparticles, Ag-NPs, *E. coli*

How to cite this article: Riah KI (2020): Bactericidal effect of silver nanoparticles and He-Ne laser on bacterium *Escherichia coli in vitro*, Ann Trop Med & Public Health; 23(S11): SP231128. DOI: http://doi.org/10.36295/ASRO.2020.231128

Introduction

The study of bactericidal nano-materials is particularly timely considering the recent increase of new resistant strains of bacteria to the most potent antibiotics [1]. Some nanoparticles are regarded as a novel approach to the development of modern pharmaceutical science which is frequently used in biology and pharmacology studies due to the high potential to perform specific treatment processes [2]. Among noble metal nanoparticles, silver nanoparticles have received considerable attention owing to their attractive physicochemical properties [3]. Silver is a non-toxic, safe inorganic antibacterial agent used for centuries and it has the capability of killing different types of diseases causing microorganisms [4].

The biological effects of low-intensity lasers are related to the exposure parameters used. High mono-chromaticity, directionality, energy densities, and laser emission mode properties are characteristics that enable semiconductor laser devices to treat various diseases [5]. He-Ne laser is highly compact, reliable. The mechanism was responsible for causing bacterial death has been reported to involve the formation of singlet oxygen and free radicals. The low-level laser has some biology effects such as cell vitality, phagocytosis, and immune responses [6]. The absorption of laser energy at low fluencies by such photo acceptors, transduction processes causing activating intracellular signaling pathways, thereby amplifying the primary photo signal [4].

Highly reactive chemical species (i.e., reactive oxygen and nitrogen species) are companies in the transduction processes, where they function as second messages, interact with biomolecules, and change cellular functions and gene expression [5]. Microbes are not able to moderate resistance against Ag-NPs, as they do against conventional antibiotics, which related to the nano-metal that attacks a broad range of targets in the organisms [7-10]. The aim of the study was to illustrate the bactericidal effect of silver nanoparticles and He-Ne laser (alone or together) on pathogenic bacterium *(E. coli)*.

Materials and Methods

Preparation of Nanoparticles

The preparation of Ag-NPswas done by laser ablation technique, where Nd-YAG laser with1064 nm wave length and maximum energy 1000 mj for each pulse. Colloidal Ag-NPs result with (20 to 40 nm) particle

size and with concentration 250 ppm(ppm= part per million), ppm equal to mg/L in the unit.The preparation was done in the Nano-technology Laboratory- College of Engineering -University of Kufa.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 representthe original and final concentrations. V1 and V2 represent the volume that need to dilute itand the final volume that result from the preparation respectively.

Preparation of Bacterial Samples

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

Helium Neon laser (2 mw)

He-Ne laser was used with 2mw power and 632.8 nm wave length has a red color, inthe medical physics laboratory-medical college - Al-QadisiyahUniversity.

Anti-bacterial effect of nanoparticles

The steps that followed for applying Ag-NPs on the bacterial species include takes tubes that contain *E.coli* 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.

Application of He-Ne laser irradiations

This was done by applying a He-Ne laser on tubes that contain *E.coli* cultured in Nutrient broth media with two irradiation times (5 and 10) minutes.

Application of He-Ne laser irradiations with Ag-NPs

This part was done byusing tubes that contain *E.coli* bacteria that cultured in Nutrient broth media with Ag-NPs in various concentrations (15, 50 and 250) ppm and then irradiate them with He-Ne laser with the sameirradiation times mentioned previously (5 and 10) min.

Elassa test

This test done by taking 0.2 ml from each tube contain E.coli bacteria that exposed to Ag-NPs only, He-Ne laser only and He-Ne laser with Ag-NPs and put them in Tissue Culture Plate (TCP) and test them by finding the absorbency for the bacteria.

Results

Nanoparticle test

The morphology and size of Ag-NPs testes were done by SEM (scanning electron microscope) Science College, Al-Kufa University and UV-Visible spectroscopy in Biology laboratory, College of Medicine, the University of Al-Qadisiyah that shown in figure (1) and figure (2).

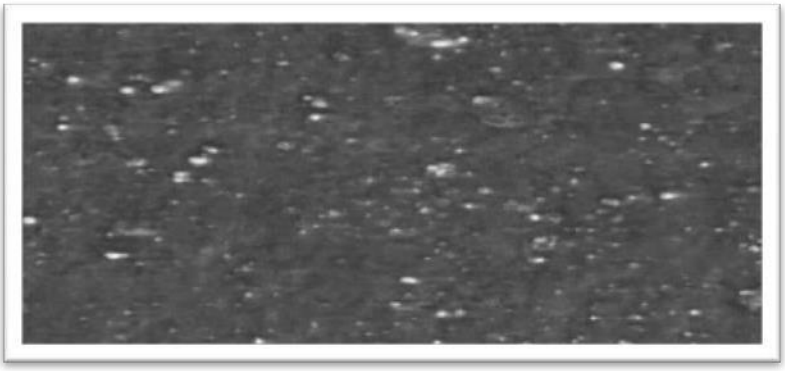

Figure (1): SEM test for Ag-NPs**.**

Effect of Ag-NPs concentrations on tested bacteria

Ag-NPs lead to inhabit the *E.coli* in all concentrations used significantly ($p > 0.05$), but there wasno significant difference between them (Fig. 3).

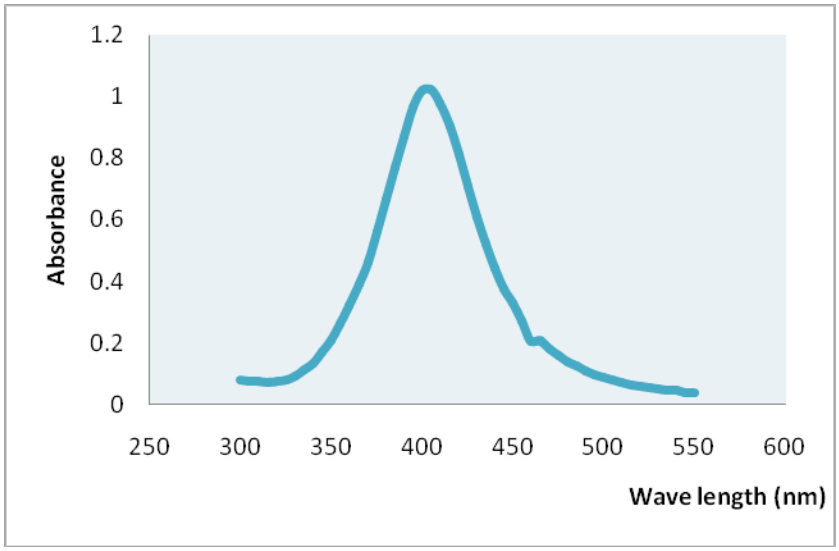

Figure (2):UV-Visible spectroscopytest for Ag-NPs.

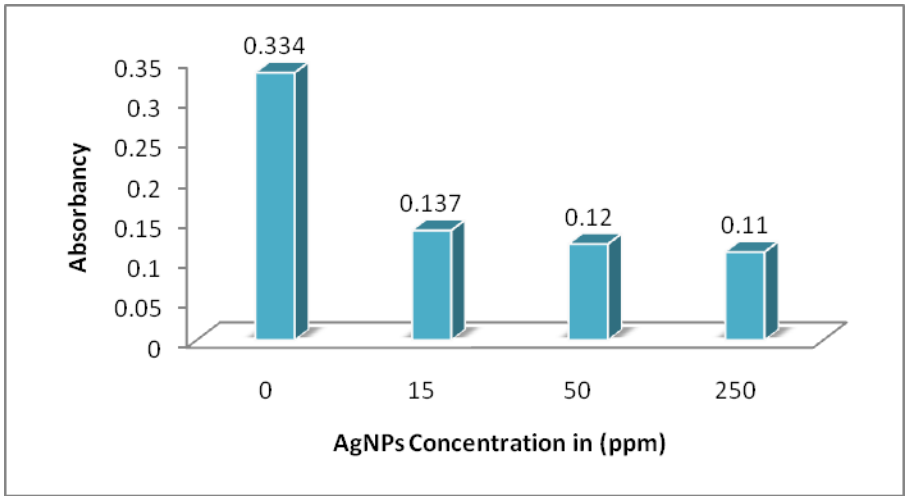

Figure (3): Effects of different Ag-NPs concentrations on *E.coli*.

He-Ne laser irradiation Effect on *E. coli* bacteria

Irradiation by He-Ne laser will cause *E. coli* inhibition significantly ($p>0.05$)as shown in figure (4).The percent of killing reach 57% from the total number in the first 5 min., but increasing the time to 10 min. will not cause any significant difference.

Combination effect of He-Ne laser irradiation and Ag-NPs concentrations on tested bacteria

The dual effect of Ag-NPs with He-Ne laser with 5 min. give more killing in *E.coli* (percent of killing more than 99%) than using He-Ne laser or Ag-NPs separately (Fig. 5). That can be shown in the first lower concentration; there was no significant difference between the other concentrations and the first concentration. The same behavior above can be shown when using more irradiation time (10 min.) as shown in figure (6).There was no significant difference in killing the *E.coli* when comparing the two irradiation times as shown in figure (7).

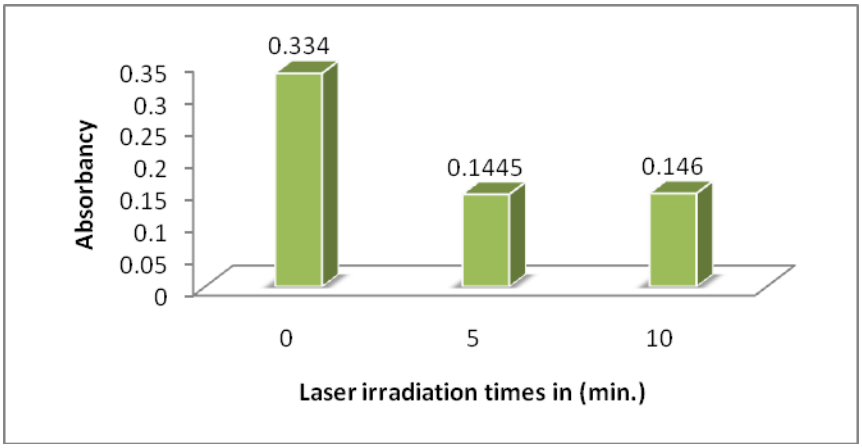

Figure (4): He-Ne laser irradiation effect on *E. coli* with different times.

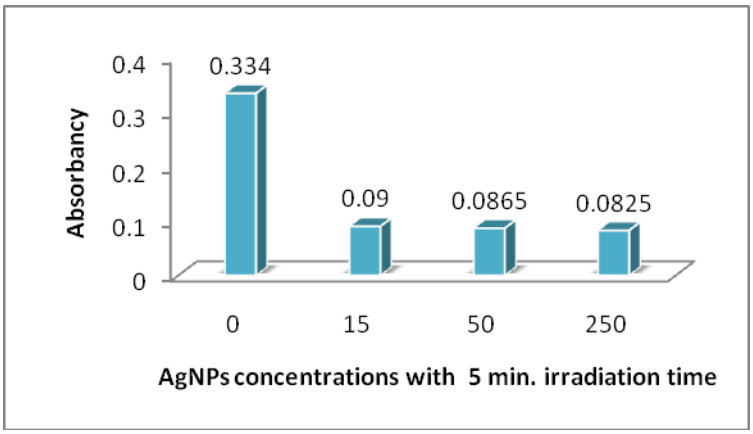

Figure (5): He-Ne laser irradiation (5 min.) with Ag-NPs effect on *E. coli.*

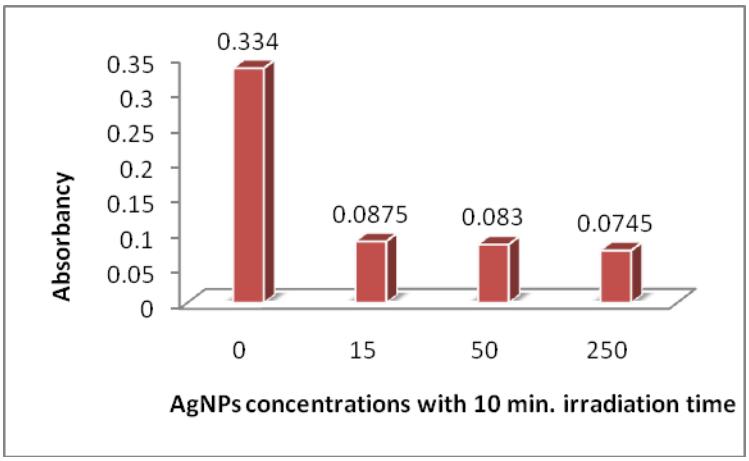

Figure (6): He-Ne laser irradiation (10 min.) with Ag-NPs effect on *E.coli.*

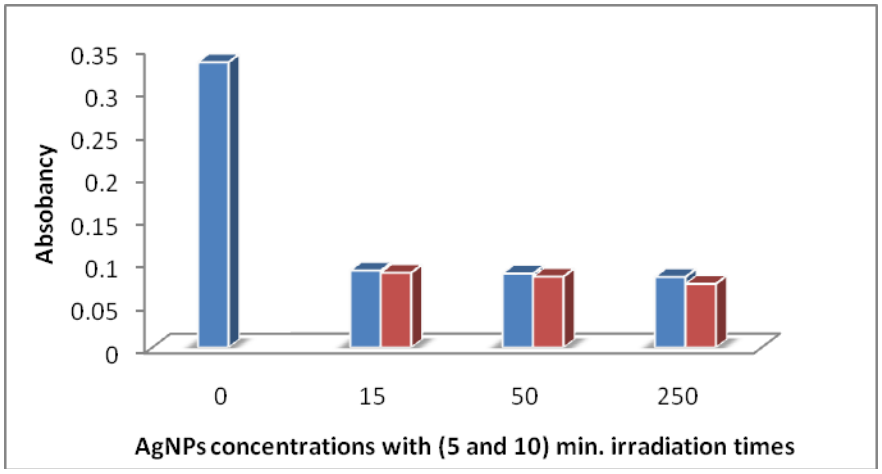

Figure (7): He-Ne laser irradiation time's effect on *E. coli*.

Discussion

Nosocomial was the most important problem in the hospital and health center around the world. The increasing bacterial resistance to antibiotics was a need for a new strategy for facing this problem; one of these was using nanoparticles as a protective factor, in addition, to using laser for enhancing bacteria killing. The silver nanoparticles show efficient antimicrobial against E. coli this may be due to the ability of these nanoparticles to attack the respiratory chain, cell division and finally leading to cell death [12, 15]. Sondhi and Salopek-Sondhi reported that Ag nanoparticles accumulated in the bacterial membrane caused a change in the permeability, resulting in cell kill [14]. In this study, the bactericidal effect of Ag-NPs used is not clearly increased with increasing concentrations and this disagrees with other research [14].

Also, increasing the time of irradiations (from 5 to 10 min.) doesn't affect the availability of *E.coli* with the power used (2 mw) and this was agreed with another study results when using He-Ne laser with 5 mw [15]. But it disagreed with other studies [16, 17] and that may be related to the limited power and irradiation time used in this study. Increasing the time of irradiation with the laser power more than that used in this study can increase the inhibition of bacteria [15]. In the present study, the combined effect of He-Ne laser and Ag-NPs influence *E.coli* bacteria and cause clear inhibition action and this disagrees with another study result, who found that the tested *S. aureus* bacteria can begin growing after 1day of incubation [18].

References

1. Jose R, Jose L, Alejandra C, Katherine H, Juan B, Jose T, and Miguel J,The bactericidal effect of silver nanoparticles. Nanotechnology 2005; 16: 2346–2353.
2. Yasaman S, Arman R. Comparing the antimicrobial effects of silver and copper nanoparticles against pathogenic and resistant bacteria of Klebsiella pneumonia, Pseudomonas aeruginosa and Staphylococcus aureus. Science Journal (CSJ) 2015, Vol. 36, No: 3 Special Issue. Iran
3. Theivasanthi T. and Alagar M. Anti-bacterial Studies of Silver Nanoparticles. (2010). Department of physics, PACR Polytechnic College, India. 1-5.
4. 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 activityEuropean Journal of Experimental Biology, 2015, 5(1):65-70
5. Barboza1 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,
6. Satturwar S, Jagtap S, Rathod V. Effects of He-Ne laser irradiation on Escherichia Coli and Bacillus subtilis. International Journal of Basic and Applied, (2012), Vol. 02: 13-19.
7. Mahmood M. The antibacterial effect of silver nanoparticles on some bacterial pathogens. Iraqi J of Physics 2012; 10:56-61.
8. Poulose S, Panda T, Nair P, Th´eodore T. Biosynthesis of silver nanoparticles. J of Nanosci and Nanotech 2014; 14: 2038–49.

9. Marambio-Jones C, Hoek E. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J of Nanoparticle Res 2010; 12:1531–51.
10. Arya V, Komal R, Kaur M, Goyal A. Silver nanoparticles as a potent antimicrobial agent: a review. Pharmacology online 2011; 3:118–24.
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 laserPhysics. Iraq. 2016, 17-18.
12. Arya, V.; Komal, R.; Kaur, M. and Goyal, A., (2011). Silver nanoparticles as a potent antimicrobial agent: a review. Pharmacology online, 3:118‑124.
13. Stadler, I.; Lanzafame, R. J.; Oskoui, P.; Zhang, R.Y.; Coleman J, and Whittaker, M., (2004). Alteration of skin temperature during low-level laser irradiation at 830 nm in a mouse model. Photomed Laser Surg, 227-231.
14. Sondi, I. and Salopek-Sondi, B., (2004). Silver nanoparticles as antimicrobial agent:a case study on *E. coli* as a model for Gram-negative bacteria. J Colloid Interface Sci., 275:177 - 82.
15. 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.
16. Raad Sh. Alnayli, Adnan H. Al Hamadani, andSafaa A. Yaseen, (2017). Silver Nanoparticles on Biosynthesis Effect of Diode Laser and Antimicrobial Escherichia Coli in Vitro. AL-Qadisiya Medical Journal Vol.13 No.24:87-93.
17. Raad Sh. Alnayli, Adnan H. Al Hamadani, andSafaa A. Yaseen, (2018). Antimicrbial Effect of Diode Laser and Biossynthesis Silver Nanoparticles on Bactrium Staphylococcus Aureus in Vitro. International Journal of Engineering & Technology, 7 (4.36) 290-292.
18. Hassan S. Physical damage to the bacteria using a combination of laser energy and absorbing nanoparticles. Advances in Environmental Biology 2015; 9: 288-94.
19. Pal S, Tak, Y, Song J. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. Appl Environ Microbiol J 2007; 73: 1712–20.