Antimicrobial Effect of Diode Laser and Biosynthesis Silver Nanoparticles on *Escherichia Coli* in Vitro

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

Address:

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

[3]Department of Physics /College of Education /University of Al-Qadisiyah,Iraq. Email: safaaameer292@gmail.com

الخلاصة

يمثل الليزر والجسيمات النانوية ملتقى العلوم البيولوجية مع العلوم الفيزيائية حيث تستخدم كمضاد للبكتيريا بدلا من المضادات الحيوية التقليدية. أعدت الدراسة كمحاولة لتوضيح كيفية استخدام الليزر أو الجسيمات النانوية للفضة كل منها على حدة، كمضاد حيوية ضد الإشريشية القولونية (*E. coli*) وكذلك دراسة النتيجة الفعالة من امتصاص طاقة الليزر بواسطة هذه الجسيمات النانوية لقتل أو تثبيط نمو البكتيريا. تم تحضير الجسيمات النانوية للفضة بطريقة بيولوجية. تم عزل *E. coli* وتمييزها باستخدام MacConkey agar والاختبارات الكيمياء- الحيوية. تم اختبار الجسيمات النانوية ضد *E. coli* باستخدام Muller Hinton agar ولكن تم اختبار أشعة الليزر (ليزرDiode) مع الجسيمات النانوية ضد *E. coli* باستخدام Nutrien broth agar. شععت الإشريشية القولونية بواسطة ليزر Diode بأزمان تشعيع مختلفة. تم استخدام تراكيز مختلفه من الفضة النانوية لقتل وتثبيط نمو البكتيريا. وأظهرت النتائج أن *E. coli* تم تثبيطها من قبل الجسيمات النانوية (بالخصوص الأعلى تركيز من الجسيمات النانوية) وأظهرت النتائج أيضا انخفاض توافر البكتيريا مع زيادة وقت التعرض لليزر.

Abstract

The laser and nanoparticles representing the concourse of biological science with physical science and considering modern method that used as an antibacterial instead of conventional antibiotics. The present study is an attempt to illustrate how the use of laser or silver nanoparticles each of them individually, as an antibacterial agent against *Escherichia coli* (*E. coli*) as well as study the effective result from the absorption of Diode laser energy by these nanoparticles to kill or inhibition bacterial growth. The silver nanoparticles (AgNPs) are prepared by biological method *E. coli* were isolated and identified by use MacConkey agar and biochemical tests. Nanoparticles are tested against *E. coli* cultured on Muller Hinton agar but Diode laser or laser with nanoparticles are tested against *E. coli* that cultured in Nutrient broth. *E. coli* are irradiating by Diode laser with different times. Different concentrations of silver nanoparticles have been employed for killing and inhibition bacterial growth. Results showed *E. coli* was effected by nanoparticles (mainly highest concentration of nanoparticles) also detected that decrease bacterial availability with increase time of exposure to Diode laser.

Key words: laser, nanoparticles, *E. coli*, AgNPs, Diode

Introduction

There is a growing concern about the emergence and re-emergence of drug-resistant pathogens such as multi-resistant *E. coli* [1]. From that, there is a needing to discover novel strategies and identify new antimicrobial agents from natural and inorganic substances to develop the next generation of drugs or agents to control microbial infections [2].Greater 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 [4-6]. The mechanism of bactericidal actions of silver nanoparticles is still not well understood but in a previous report [7] 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[8,9]. 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 [7,11]. 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]. So, the aim of this study was to evaluate the effect of silver nanoparticles and laser Diode on the growth of the gram negative bacteria E-coli isolated from hospitalized infections.

Materials and Methods

Preparation of nanoparticles

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

Preparation of Bacterial Samples:

The bacterial sample of *E. coli* was collected from the Central Health Laboratory from Al Najaf city, which is taken from the surgical tools in the operation rooms of the Al- Najaf teaching hospital. The collection was done by swab and then *E. coli* were isolated and identified by use MacConkey agar and biochemical tests.

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. 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 250C 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 number113.

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 25ºC in dark along with control 13. Colloidal nano silver that has particle size 20 nm to 40 nm (average = 30 nm) with different concentration (36.21ppm) that equal to mg/L (This concentration was diluted to one half and quarter).

Bacterial susceptibility to nanoparticles.

To examine the susceptibility of *E. coli* 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 was measured manually.

Application Laser Irradiations on Bacterial Species:

The procedure for applying Diode laser (with 50 mw) on the tubes that contain *E. coli* cultured in Nutrient broth and irradiate them with Diode laser with different irradiation times (1,3,5,7,10,15,20) minutes.

Application Laser with nanoparticles on Bacterial Species:

The procedure for applying the Diode laser on the tubes that contain *E. coli* bacteria cultured in Nutrient broth with different concentrations of AgNPs and

irradiate them with Diode laser with different irradiation times (1,3,5,7,10) minutes.

Results

Nanoparticle test

The size and morphology of nanoparticles were determined by UV-Vis spectroscopy and scanning electron microscope (SEM), as seen in figure (1) and figure (2).

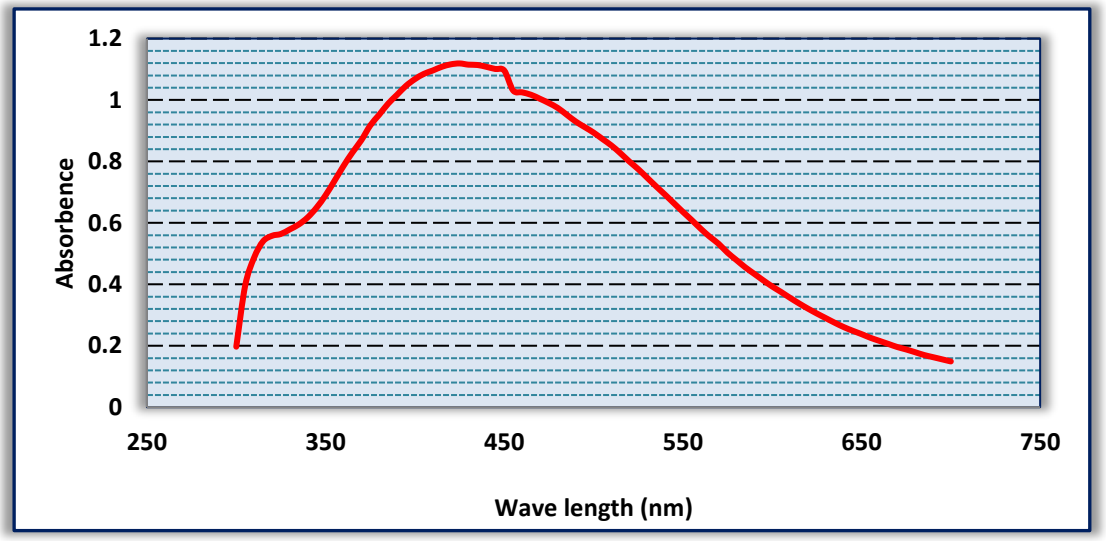

Figure (1): Absorption spectrum for biosynthesis of AgNPs.

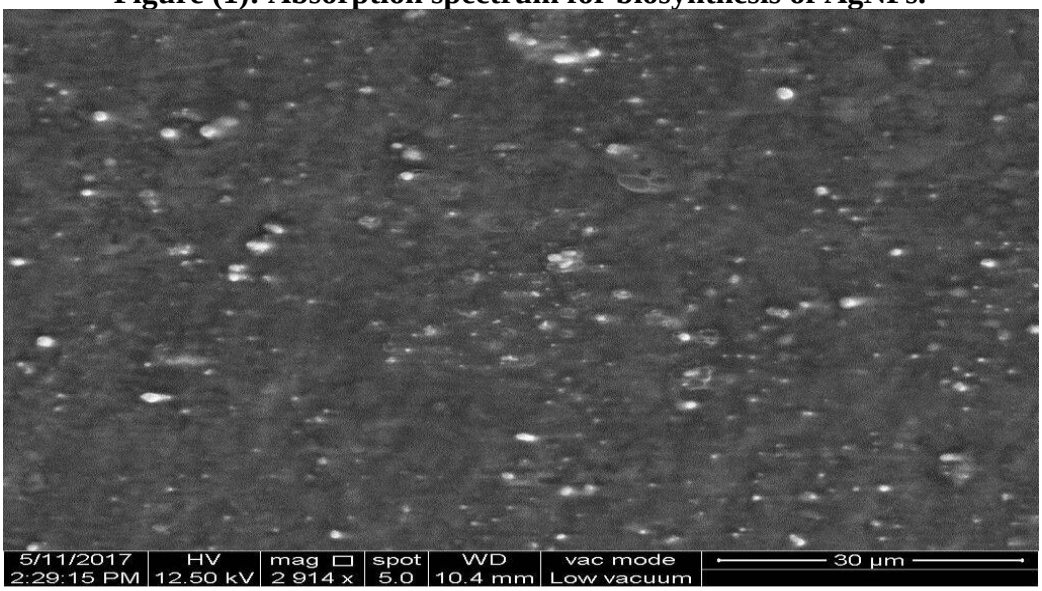

Figure (2): SEM test for biosynthesis of AgNPs.

Antimicrobial Effect of Nanoparticles on *E. coli*

The effect of AgNPs on *E. coli* growth can be seen in figure (3). Results showed that sensitivity of *E. coli* to nanoparticles increased with increasing concentration of nanoparticles (figure 4).

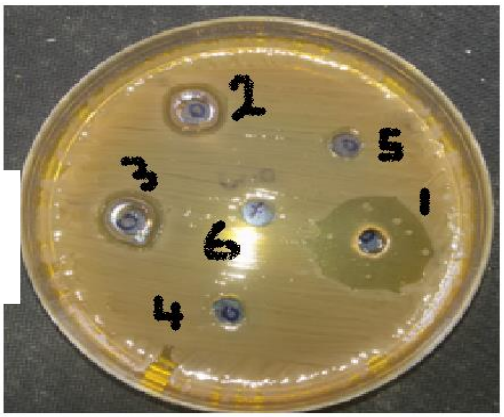

Figure (3): Image for effect of AgNPs on *E. coli* that cultured on Muller Hinton agar. Where**:** 1,2,3 represent concentrations of biosynthesized AgNPs with 36.21 ppm, 18.1 ppm and 9 ppm

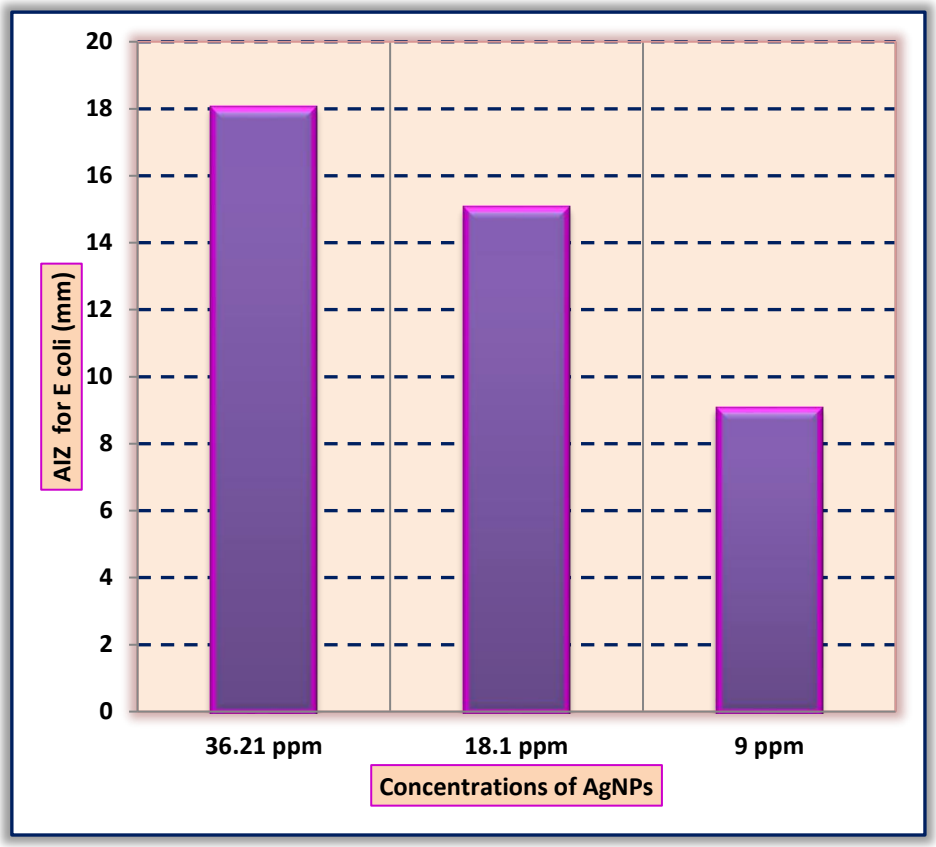

Figure (4): Average diameter measured for effect of AgNPs in different concentrations on *E. coli.*

Effect of Laser Irradiations on E-coli growth

The effect of Diode laser (50 mw)in different irradiation times on *E. coli* can be seen in the figure (5) that showed the increases irradiations time associated with decrease bacterial availability in Nutrient broth (low absorbency)

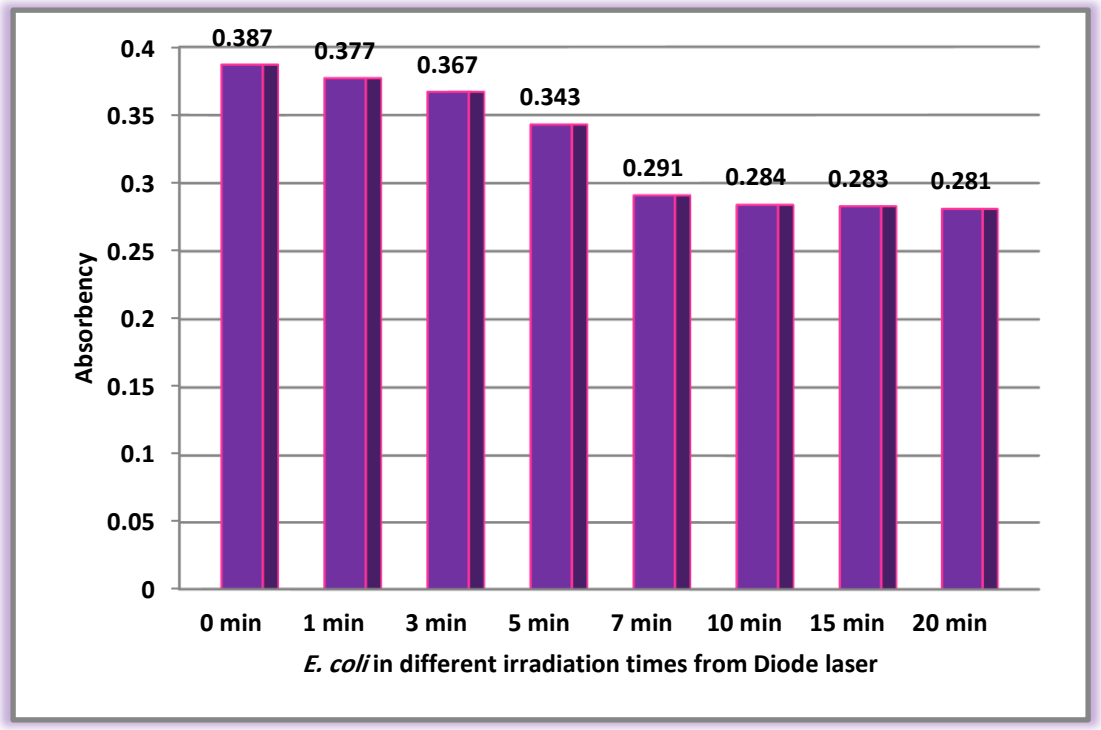

Figure (5): Effect of Diode laser in different irradiation times on *E. coli* cultured in nutrient broth.

Mutual effect of laser and nanoparticles on E-coli growth

Figures (6) showed the effect of Diode laser (50 mw) with AgNPs on *E. coli.* Although the effect of Bacteria by dual action of Laser Irradiation with Nanoparticles are very little, the inhibitory effect mainly appeared at long Irradiation time (10 min) with highest concentration of nanoparticles.

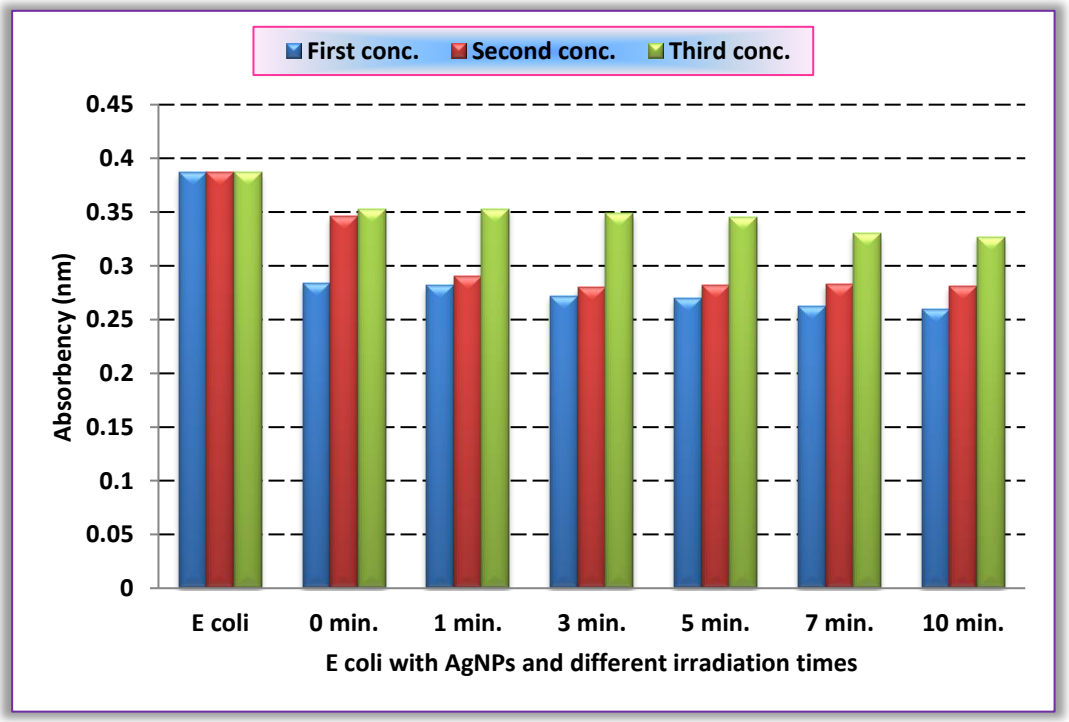

Figure (6): Effect of Diode laser in different irradiation times with AgNPs in different concentrations on *E.coli* cultured in nutrient broth.

Discussion

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 [11, 13]. Some studies have reported that the positive charge on the Ag ion is crucial for its antimicrobial activity through the electrostatic attract yu ion between negative charged cell membrane of microorganism and positive charged nanoparticles [7,8,12]. Sondi and Salopek-Sondi reported that Ag nanoparticles accumulated in the bacterial membrane caused the permeability, resulting in cell death [13]. Amro *et al* suggested that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by progressive release of lipopolysaccharide molecules and membrane Proteins [14]. In this study antimicrobial action of used nanoparticles is enhanced with increase their concentration and this agree with other studies [2,15,16]. Theivasanthi and Alagar showed that the toxicity of silver nanospheres is higher than that of gold nanospheres and bacteria not able to develop immunity to silver as they often do with antibiotics [14]. Furthermore, Pal *et al*. found that interaction of nanoparticles with *E. coli* shape dependent, since truncated triangular particles showed higher activity compared to spherical and rod-spherical particles [5].Current study detected inhibitory action of laser on *E. coli* and this agree with study of Rathod *et al* (2012) who show that exposure of bacterial cultures to Diode laser light results in a decrease in viability and suggest that power of laser and time of exposure plays an important role in its efficacy [7]. The mechanism of laser-induced cell destruction has important implications in clinical therapy. According to Karu exposing a cell to laser light causes acceleration of electron transfer in some areas of the respiratory chain. At higher doses, this excitation energy is transferred to oxygen to form oxygen radical [12]. In general, the ability of the laser light to kill the micro-organisms is species dependent. The reasons contributing to this observation remain unknown. One view suggests the role of cell morphology, especially the kind of pigmentation of the cell wall that determines the susceptibility of the different bacterial species to laser radiation [7]. In the study current, the dual action of laser and nanoparticles not have clear inhibitory action on *E. coli* and this agree with study of Hassan, (2015) who determine that tested *E. coli* can re-grow after 24 hr of incubation [16].

Conclusion

The inhibition or killing of bacteria was increased when the concentrations of nanoparticles were amplified. In addition, sensitivity of E. *coli* to laser enhance with increase time of exposure.

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