Antibacterial Effect of Zinc Oxide Nanoparticles and He-Ne Laser on Pseudomonas Aeruginosa in Vitro

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Abstract

This work was designed to study the effect of He-Ne laser (2 mw) and Zinc Oxide nanoparticles (ZnO-NPs) each of them separately on Pseudomonas aeruginosa. Also, illustrate the combined action of He-Ne laser and ZnO-NPs on this bacterium. Two different irradiation times for laser were used and three different concentrations of ZnO-NPs were applied to observe the inhibition on bacterium. The results show increase bacterium inhibition in the high concentration of ZnO-NPs used in this study. He-Ne laser irradiation times with (5 and 10 min.) do not inflict bacterial availability when used alone or with ZnO-NPs.

Key words: He-Ne laser, ZnO-NPs, Nanoparticles, Pseudomonas Aeruginosa.

DOI Number: 10.14704/nq.2020.18.8.NQ20209 **NeuroQuantology 2020; 18(8):86-92**

Introduction

Widespread usage of antibiotics and bacterial resistance to antibiotics has become a universe problem nowadays, and this encourages scientists around the world to develop newer versions of antimicrobial agents such as metal oxide nanoparticles [1, 2].

Nanoparticles have unique features such as surface Plasmon absorption and enhanced catalytic activity because of their quantum size confinements and large surface areas [3-6]. Zinc oxide nanoparticles (ZnO- NPs) application as antibacterial agent stands out in comparison to other mineral nanoparticles [7]. ZnO-NPs are reported to have bactericidal action, and they can reduce skin infection and improve infected skin architecture [8,9].

ZnO-NPs main bactericidal mechanisms due to the induction of oxidative as a result to the formation of reactive oxygen species and membrane disturbance related to the accumulation of ZnO-NPs inside [10,11]. Zn are also described to destroy bacterial membranes in their nanoparticle forms [2].

In addition to its properties mentioned above, ZnO is considered as a Generally Recognized as Safe (GRAS) compound by the U.S. Food and Drug Administration [12].

Laser beam has covered the significant aspect of life including medicine and biology [13-15]. Laser therapy is a noninvasive treatment with many impacts and applications, including on each of the stages of cells healing [16-19].

Low-level laser (LLL) has some biological effects such as cell vitality and phagocytosis; also it's used for the treatment of some diseases [20, 21]. LLL is a type of monochromatic light source in the range of (1-1000) mw [22].

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 18 June 2020 **Accepted:** 24 July 2020

He-Ne is a type of gas laser operates at a wavelength of 632.8 nm in the red part of visible spectrum. It has spatial characteristics such as high intensity, good directivity, good monochromatic and coherence. It is a variable low-level laser [23-26]. The mechanism of He-Ne laser responsible for causing death of bacteria has been reported to involve the acceleration of electron in some part of respiratory chain causing formation of singlet oxygen and free radicals lead to killing of microorganisms [27, 28].

Pseudomonas aeruginosa (P. aeruginosa) is gram negative bacteria, motile and rod shaped measuring about 0.6x2µm. It is one of the most common bacteria encountered in hospital infection. It was isolated from various sources like air, floor, sinks and even disinfectant. Also, it's frequently present in small numbers as normal intestinal flora and on the skin of humans. The most noticeable properties of *P. aeruginosa* are its natural resistance to antibiotics and disinfectants [29].

The Aim of the Study

The aim of this study was to observe the impact of ZnO-nanoparticles and He-Ne laser (alone or with each other) on *Pseudomonas aeruginosa*.

Material and Method

1) Bacterium

Pseudomonas aeruginosa bacterium sample were obtained from the surgical appliances in the surgical rooms of hospital in Al-Najaf city, then, they were tested, isolated and identified in the Central Health Lab. in the same city.

2) ZnO-NPs Production

Acetate zinc was dissolved in a mixture of mono-ethanol secretary and methanol at room temperature and mixed by a magnetic mixer for 60 min. until the mixture get a homogenous solution, then left for 24 hours, after that, at temperature of 200°C, the solution (mixture) was heated for three hours, then, at temperature of 500°C the black material precipitate calcined, after that, a white powder was collected (Zinc oxide nanoparticles).

3) Preparation of ZnO-NPs Concentration

This done by dissolving 10 mg of Zno-NPs in 10 ml of dimethyl sulfoxide solution (DMSO) getting stock solution of 1 mg/ml, after that, dilution 1 ml of this solution by 10 ml of DMSO again getting a solution with a concentration of 100 µg/ml. The required concentration (25, 50, 75 µg/ml) for this study had been prepared from this solution [30].

4) The Application of ZnO-NPs on Bacterium

The ZnO-NPs were applied in the following procedure:

A- The prepared sub-culture bacteria were placed in nutrient broth media tubes.

B- Tow 0.2 ml of colloidal ZnO-NPs (with the three prepared concentrations) and placed in each tube.

C- The tubes were incubated for 24 hours with 37°C.

D- The bacterial inhibition was determined by ELISA test.

5) Application of He-Ne (2 mw) laser irradiations

He-Ne laser was used (632.8 nm wave length and red colour) with 2 mw power was applied on tubes that contains Nutrient broth media with *Pseudomonas aeruginosa.* It was exposed to two irradiation times (5 and 10) minutes.

6) Application of He-Ne Laser on Bacterium with ZnO-NPs

This done by irradiation the tubes that contain the bacterium that cultured in Nutrient broth media with ZnO nanoparticles in different concentrations (25, 50 and 75) µg/ml. The irradiation times were the same that mentioned previously.

7) Elassa Test

This part was done by taking 0.2 ml from each tube contain *Pseudomonas aeruginosa* bacterium that exposed to ZnO- NPs only, He-Ne laser only and He-Ne laser with ZnO-NPs, then placed in Tissue Culture Plate (TCP). The test was run after put the TCP in the device and give result by measuring the absorbency for each sample.

The Results

1) Nanoparticle Test

The purity and crystalline spectrum and ZnO-NPs can be shown in figure (1) that done with Energy Dispersive X-Ray spectroscopy (EDS). Purity of ZnO-NPs is 100% where, there were no impurities appears in the spectrum.

The morphology and size determination of ZnO-

NPs were done by scanning electron microscope (SEM) as shown in figure (2).

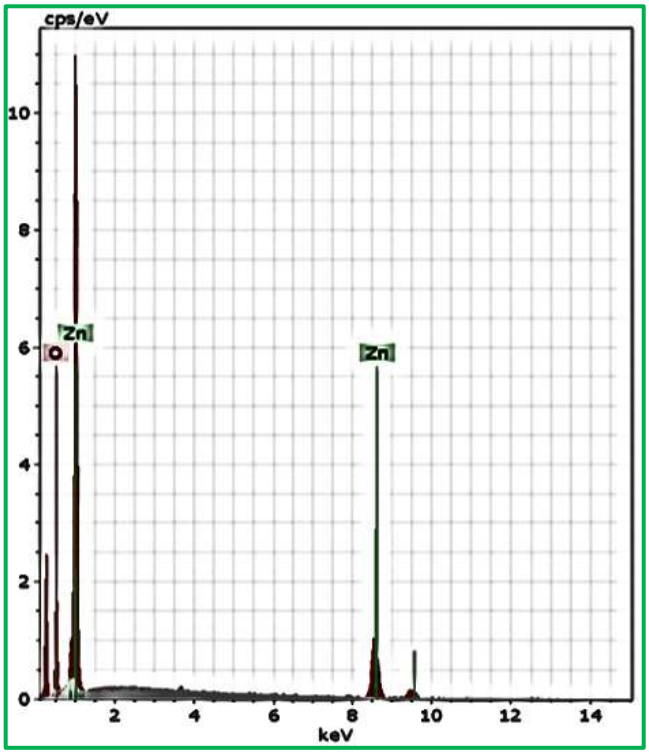

Figure 1. Energy Dispersive X-Ray spectroscopy spectrum of modified ZnO-NPs

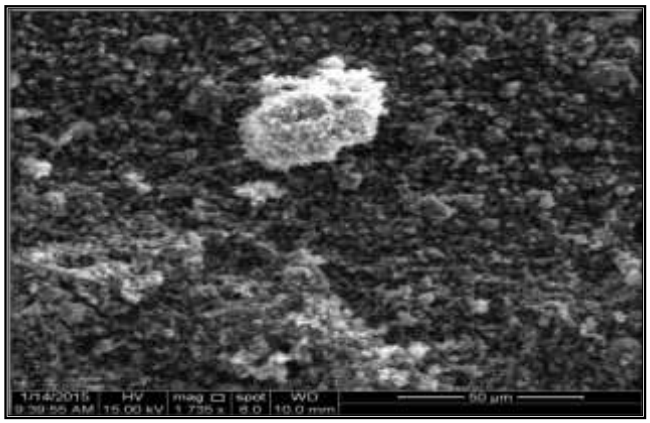

Figure 2. Scanning electron microscope test for ZnO-NPs with various magnifications

2) *Effect of ZnO-NPs on Tested Bacterium*

The first concentration (75 µg/ml) has significant effect ($p < 0.05$) on *Pseudomonas aeruginosa* bacterium while the third and second concentration (25 and 50 µg/ml) does not affect significantly as seen in figure (3). There was low effect of 25 µg/ml in inhibition of the bacterium when compared with two other concentrations, so, will be excluded in the next parts of the results.

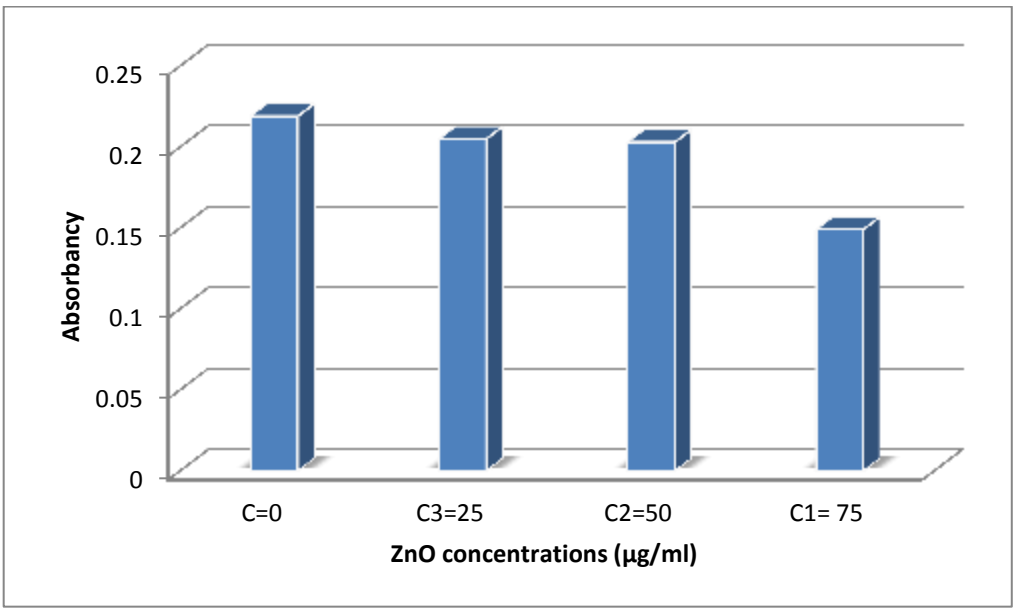

Figure 3. Effect of ZnO-NPs concentrations on Pseudomonas aeruginosa bacterium

3) *Effect of He-Ne Laser Irradiation Times on Tested Bacterium*

There was no significant effect of the first irradiation time (5 min.) and second irradiation time (10 min.) on tested bacterium. That can be seen in figure (4).

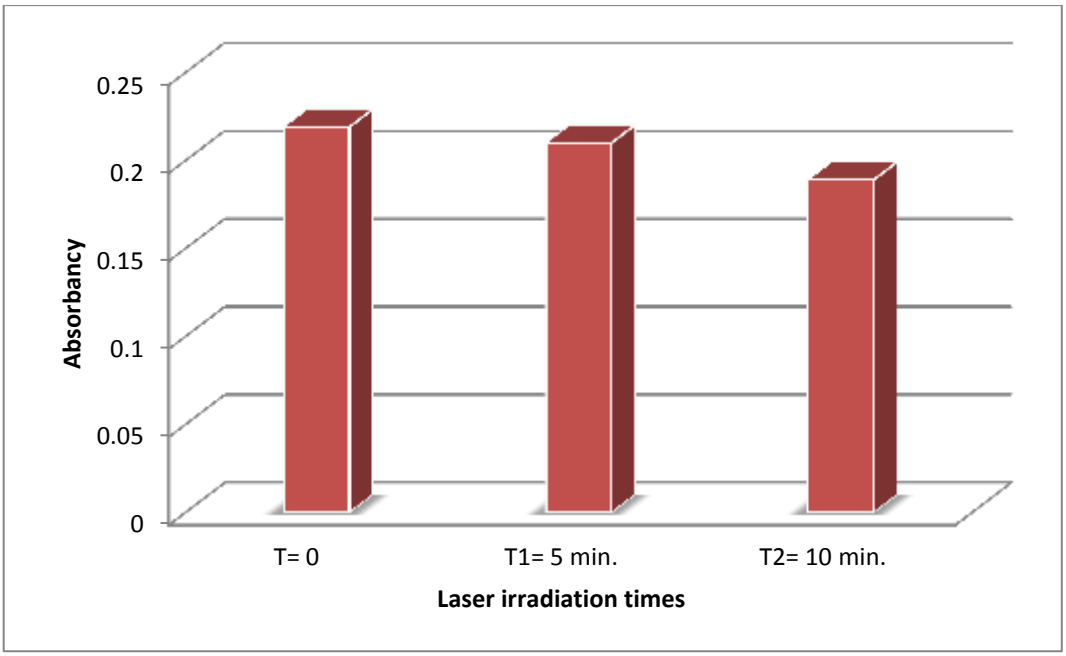

Figure 4. Effect of He-Ne laser irradiation times on tested bacterium

4) Synergetic Effect of ZnO-NPs and Laser on Tested Bacterium

The mutual effect of ZnO-NPs with 5 min. irradiation time by He-Ne laser doesn't increase the inhibition of *Pseudomonas aeruginosa* (figure 5) when compared with the effect ZnO-NPs only (figure 3). So the bacterium reduction shown in figure (5) was related to the effect of nanoparticles only.

Also, using 10 min. irradiation time (figure 6) does not give enhancement in killing of the bacterium and the inhibition shown was related to the nanoparticles.

There was no significant difference between the two irradiation times with the existing of ZnO-NPs on the bacterium as seen in figure (7).

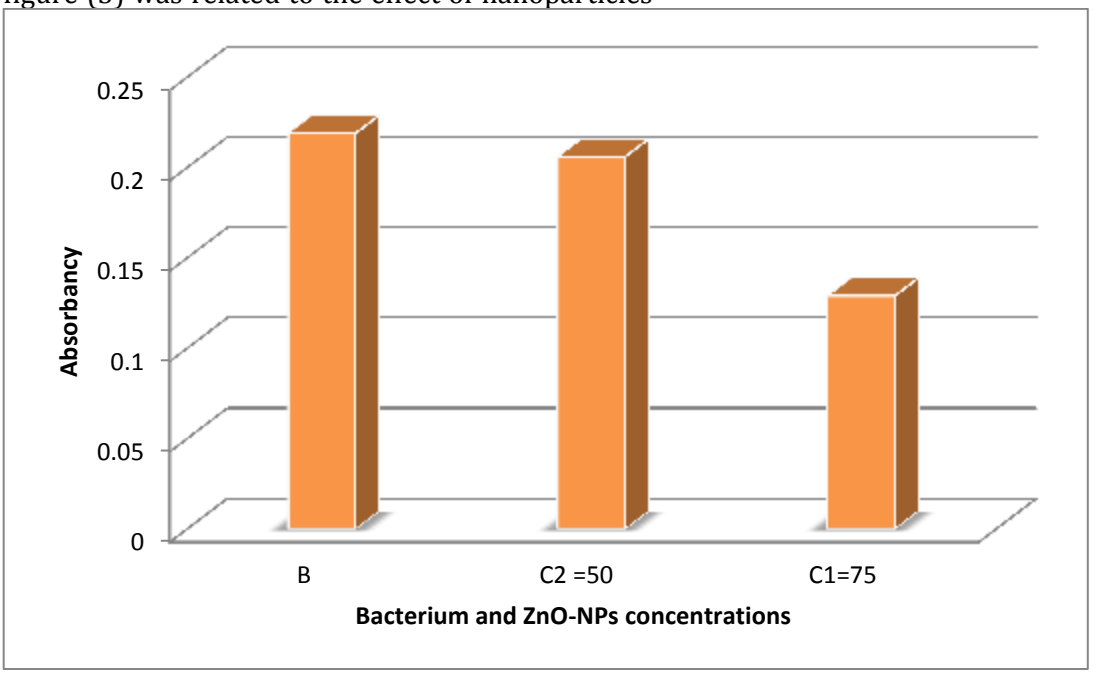

Figure 5. Effect of ZnO-NPs and 5 min. He-Ne laser irradiation time on tested bacterium

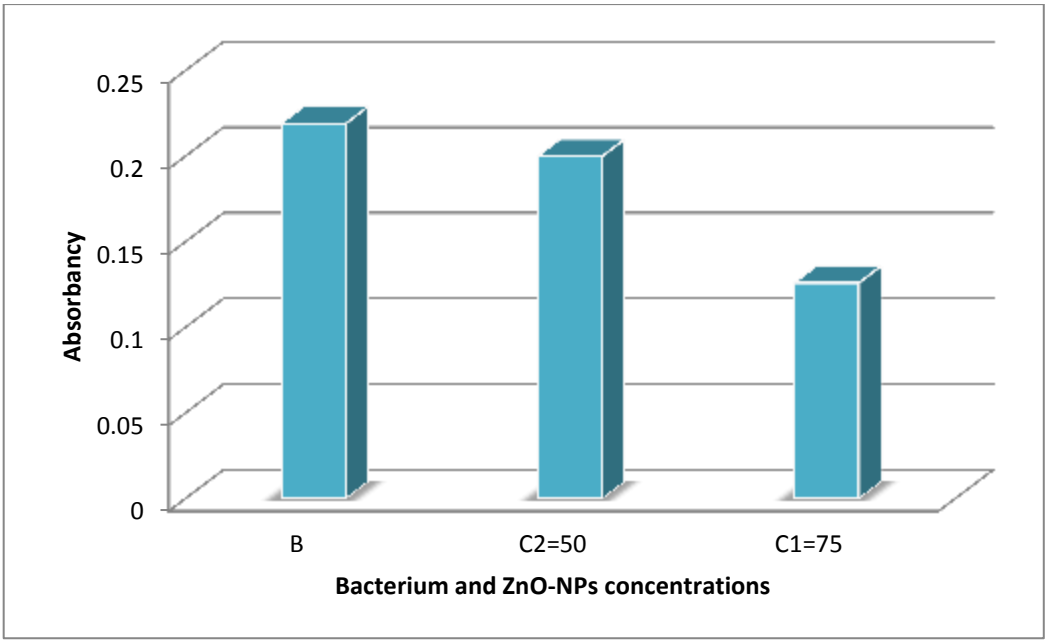

Figure 6. Effect of ZnO-NPs and 10 min. He-Ne laser irradiation time on tested bacterium

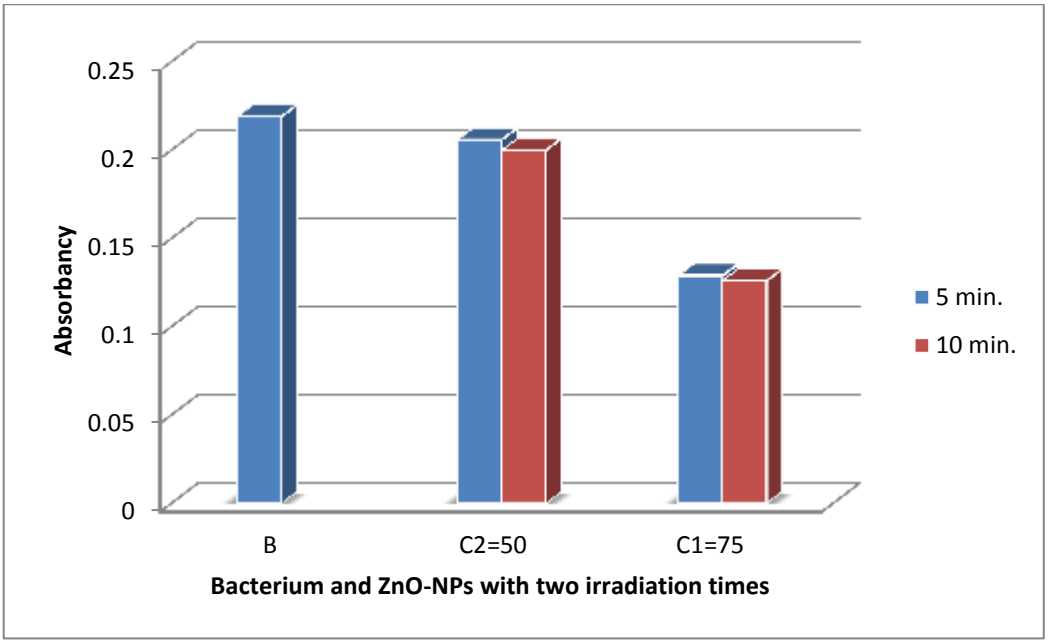

Figure 7. Effect of ZnO-NPs and He-Ne laser irradiation times on tested bacterium

Discussion

In this study, bacterial inhibition was gained when using high concentration (75 µg/ml) and below this concentration there were no significant effect.

Other researchers found that the effective concentration of ZnO-NPs (that modified with an oleic acid) was 40 µg/ml for killing *P. aeruginosa* [30].

Unexpected results were shown when the absorption of laser energy by bacterium alone or with existing of ZnO nanoparticles doesn't increase the inhibition of bacterial growth. This may related to the low power used (2 mw) and the times depend for irradiation (5 and 10 min.).

Study results related to the He-Ne laser don't has acceptance with other studies that used other types of bacteria, the inflict of He-Ne laser on *Bacillus subtilis* and *E coli* shows that reduce in viability at effective combination of 7.5 mw and 90 sec. [31].

Another study reported that two various dosages of He-Ne laser with power (3 and 5) mw at different times resulted in various effects. The better effect was 5 mw with 180 sec. in case of *Pseudomonas aeruginosa* [13].

Furthermore, using laser with more power like using Diode laser with 50mw will reduce the time required to induce bacterial inhibition [32].

Conclusion

1) Increasing the concentration of ZnO-NPs till reach 75 µg/ml cause inhibition of *P. aeruginosa.*
2) He-Ne laser has no effect on the bacterium when used alone or in combination with nano ZnO due to the low power used (2mw).

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