Effect of He-Ne Laser and Zinc Oxide Nanoparticles on Pathogenic Bacterium Escherichia Coli in Vitro

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Abstract

This study illustrate the effect of Zinc oxide nanoparticles (ZnO-NPs) or He-Ne laser each of them individually, on Escherichia Coli (E. coli), also study the dual effect of nanoparticles with various concentrations and laser energy with two different irradiation times to kill or inhibition of bacterial growth. The results showed that E. coli was affected by ZnO-NPs in high concentration and there was a little effect of laser irradiation with 2 mw power when used alone or when used together with ZnO-NPs.

Key Words: Laser, He-Ne, Nanoparticles, ZnO-NPs, E.coli.

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Introduction

Antibacterial was the preferred treatment method for bacterial infections due to their low cost and good outcomes [1]. However, bacterial resistance to antibiotics has become a global problem nowadays. In examining different options to solve this problem, nano-materials, such as metal oxide nanoparticles, have appeared to be promising candidates during the last few years [2]. Nanoscale reduction in size can change their structural, morphological, optical, chemical, electrical and mechanical properties. These modified properties make easy the physical transfer of nanoparticles into living cells and allow them to interact with various biomolecules in the cell [3].

Nanoparticles are one of the very important aspects that applied in medical field as the biological processes appear at nano-scale and nanoparticles have the ability to amend the cellular biological function of the cells [4]. It was appeared in the modern studies that metal oxides nanoparticles, such as Zinc oxide (ZnO), have the ability to select amount of toxicity to different species of bacteria but have low effect for the human cells [5-8].

ZnO nanoparticles were reported by some recent studies have no toxicity to human body, this aspect imposed their use as antimicrobial factor, harmful to micro-organisms, and hold bio-compatibility to cellular environment of human body [9, 10]. The mechanism of ZnO-NPs toxicity depends on the fundamental physiochemical properties, modified properties of the surface of ZnO, and the used medium for dispersion of the nanoparticles [11]. ZnO- nanoparticles seen to change the gram negative cellular structure of the membrane in *Escherichia coli (E coli)* [5], and it was suggested that the gram-negative cell membrane could bind nano-particles with a positive charge such as cerium oxide by electrostatic attraction [12].

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Low level lasers (LLL) are light weight, available sources of non-ionizing radiation, monochromatic, practical and low-cost. For these reasons, these devices have increasing usage in medicine [13].
Although low level laser treatment (LLLT) has been widely used, many recent researchers have shown little or no benefit of LLLT. The physiological mechanism of LLL is poorly understood, and treatment parameters such as intensity, wavelength, frequency, and dosage are uncertain as well [14,15]. He-Ne laser has spatial characteristics such as 632.8 nm of wavelength, directionality, high intensity, high mono-chromaticity and coherence is a variable low-level laser [16-19]. The low-level laser has some biology effects such as cell vitality [20].

The Aim of the Study

The coal of this study was to illustrate the effect of ZnO-nanoparticles and He-Ne laser (alone or together) on pathogenic bacterium *E. coli*.

Materials and Methods

1) Bacterium

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

2) Production of ZnO-NPs

Acetate zinc was dissolved in a mixture of mono-ethanol secretary and methanol at room temperature and by a magnetic mixer was mixed for 1 hr. until the mixture have been a homogenous solution, then left for 1 day, after that, at 200°C, the solution was heated for 3 hrs., then, at 500°C the black material precipitate calcined, after that, they was collected a white powder (nanoparticles zinc oxide).

ZnO-NPs Concentration Preparation

Dissolving 10 mg of Zno-NPs in 10 ml of dimethyl sulfoxide solution (DMSO) getting stock solutions of 1 mg/ml, then dilution 1 ml of this solution by 10 ml of DMSO again yielding a concentration of a solution with of 100 µg/ml. The required concentration for this study which include: 25, 50, 75 µg/ml (ppm) had been prepared from this solution [21].

3) Application of ZnO-NPs on Bacterium

The ZnO-NPs were applied as follows:

1. Sub-culture (prepared previously) was placed in nutrient broth media tubes.
2. Taking 0.2 ml of colloidal ZnO-NPs (with the prepared concentrations) and placed in each tubes.
3. These tubes were incubated for 1 day with 37°C temperature.
4. Determine the bacterial inhibition in the ELISA test.

4) Application of He-Ne (2 mw) Laser Irradiations

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

5) Application of He-Ne Laser Irradiations with ZnO-NPs

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

6) Elassa Test

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

The Results

1) Nanoparticle Test

The morphology and size of ZnO-NPs testes were done by SEM (scanning electron microscope) (Fig.1).
The crystalline ZnO-NPs spectrum and the purity can be shown in figure (2) that done with Energy Dispersive X-Ray spectroscopy (EDS).Also, the purity of ZnO is 100% since there were no impurities appears in the spectrum.

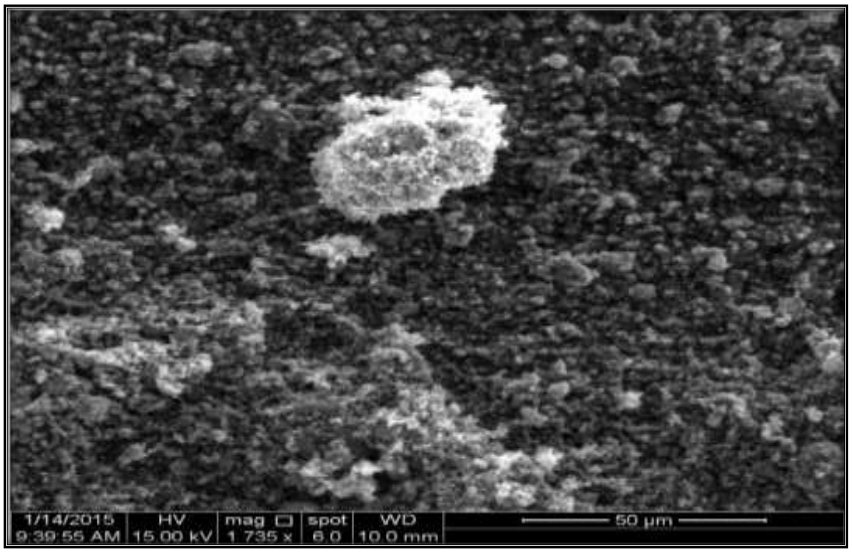

Figure 1. SEM test for ZnO-NPs with different magnification

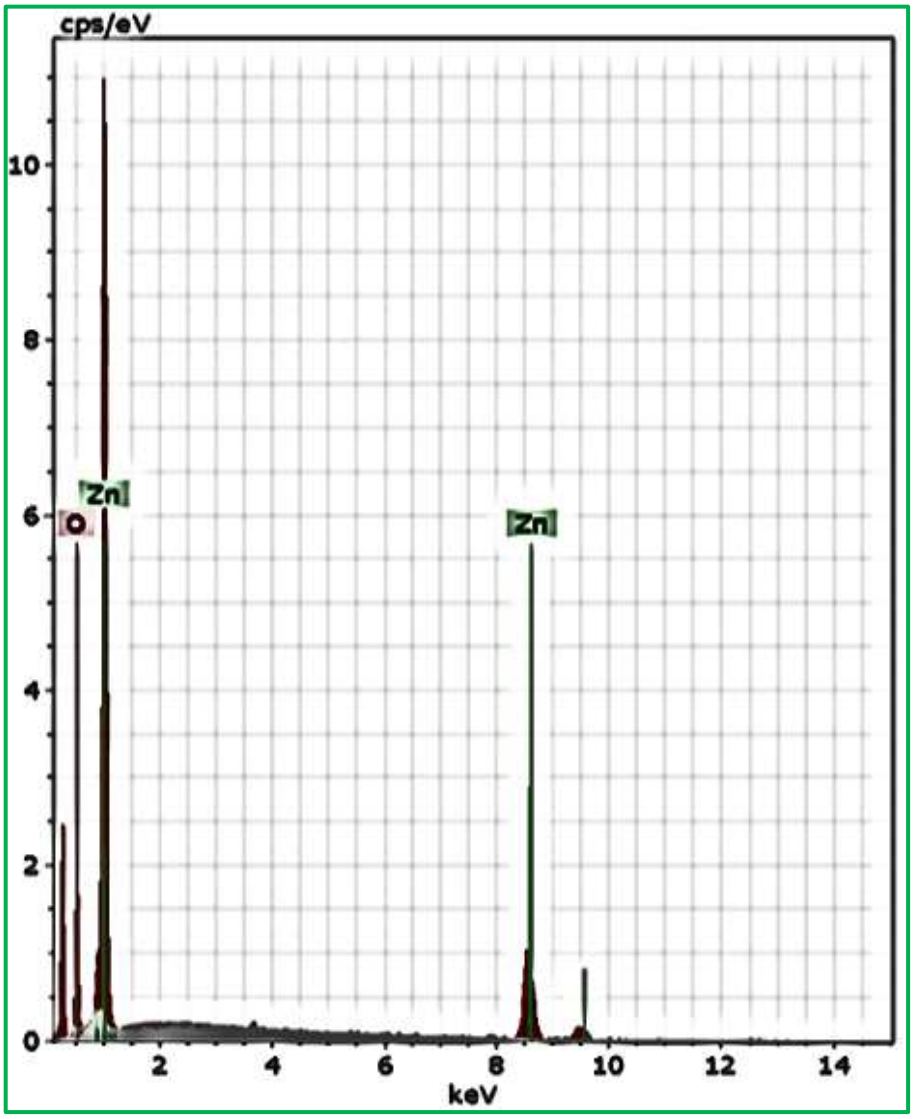

Figure 2. EDS spectrum of modified ZnO NPs

2) ZnO-NPs Concentrations Effect on Tested Bacterium

ZnO-NPs don't effect significantly on *E coli* with first and second concen-trations. The third concentration has significant effect ($p < 0.05$) on *E.coli* (Fig. 3).

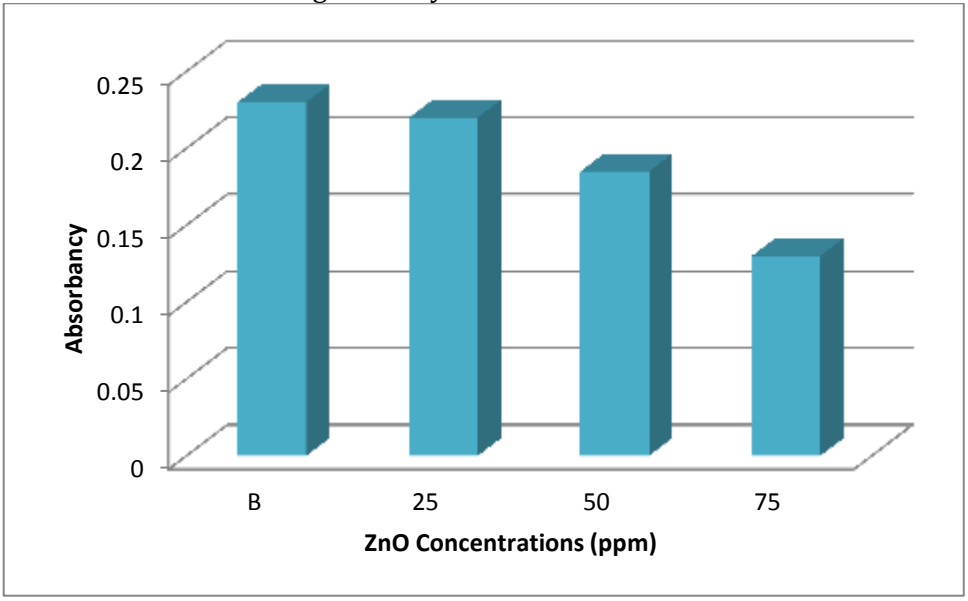

Figure 3. Effects of different ZnO-NPs concentrations on E.coli

3) He-Ne Laser Irradiation Effect on E.coli Bacteria

Irradiation by He-Ne laser with 5 min. will cause minimal effect on *E.coli* and this effect was increased when increase the time of irradiation (10 min.) as shown in figure (4). But, the two irradiation times don't cause any significant difference on this bacterium.

4) Combination Effect of Irradiation by He-Ne Laser and ZnO-NPs Concentrations on Tested Bacterium

The synergetic effect of ZnO-NPs with He-Ne laser with 5 min. doesn't cause clear change in the reduction of *E.coli* (Fig. 5) when compared with using ZnO-NPs alone (Fig.3). So the bacterium inhibition shown in figure (5) was related to the effect of ZnO-NPs only.

The same effect above (figure-5) can be shown when using more irradiation time (10 min.) as shown in figure (6).

There was no significant difference in inhibition of *E.coli* when comparing the two irradiation times (Fig.7).

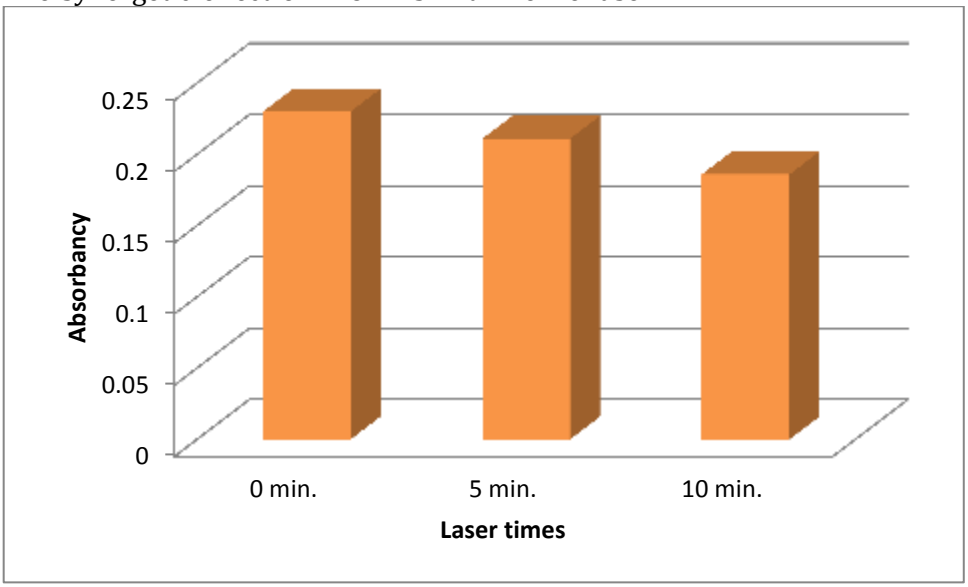

Figure 4. He-Ne laser effect on E.coli with two irradiation times

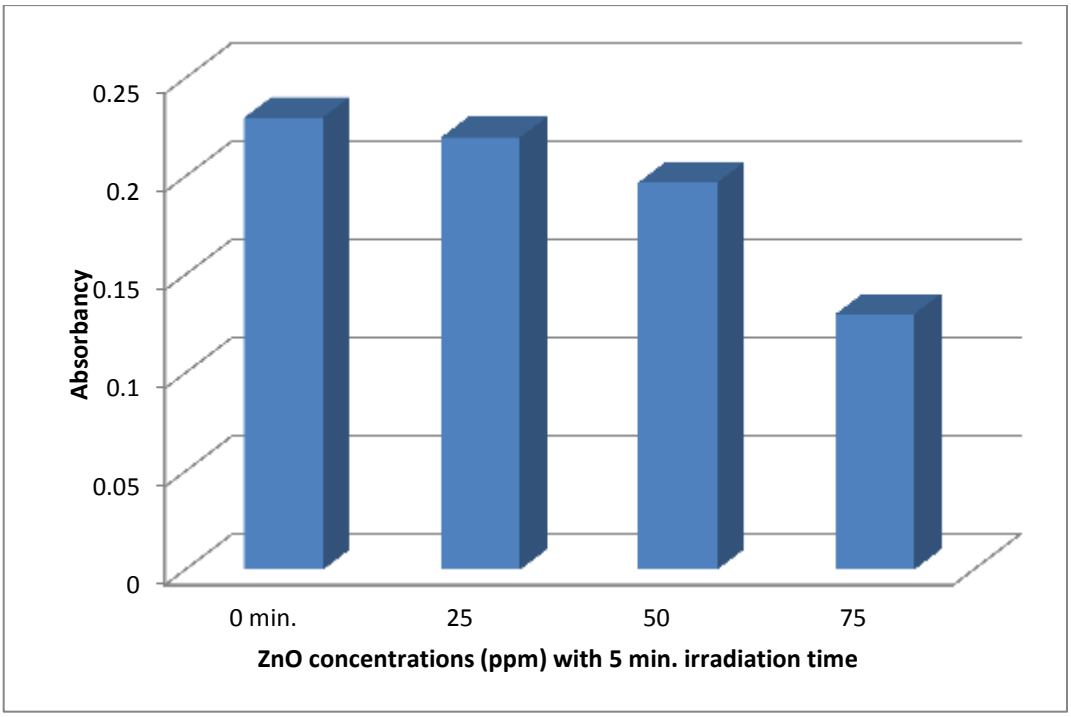

Figure 5. Effects of Irradiation by laser (5 min.) and ZnO-NPs concentrations on E.coli

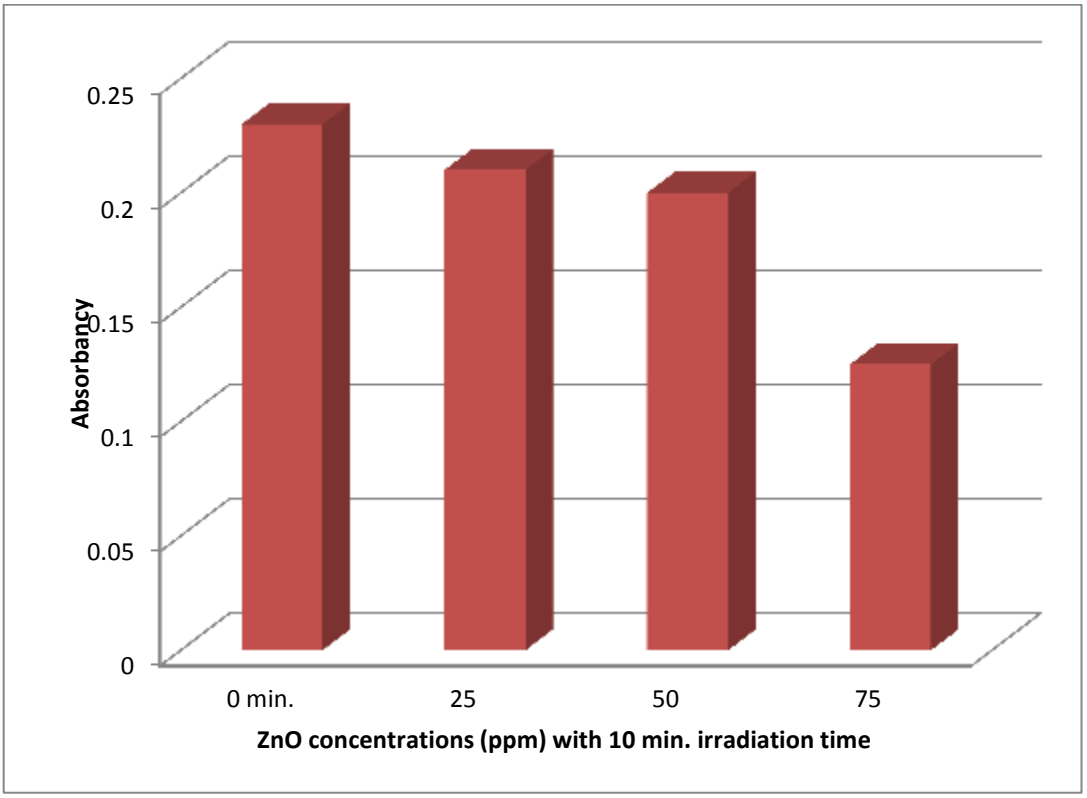

Figure 6. Effects of irradiation by laser (10 min.) and ZnO-NPs concentrations on E.coli

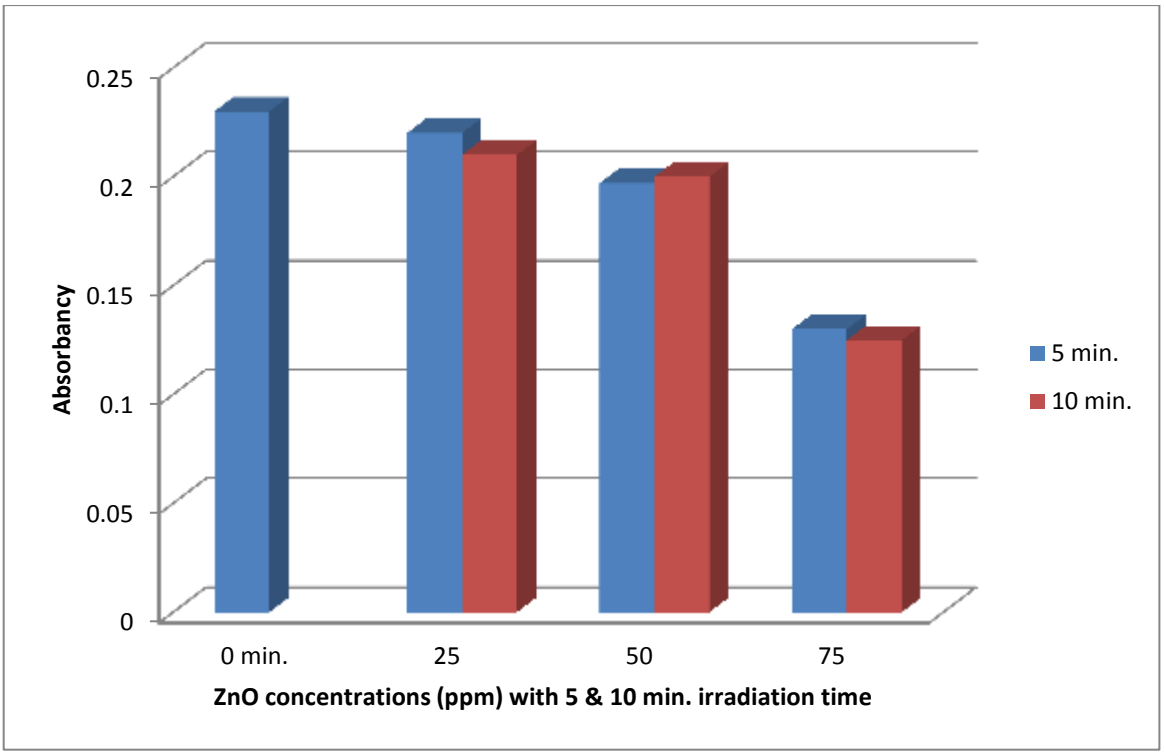

Figure 7. Effects of ZnO-NPs concentrations with laser irradiation times on E.coli

Discussion and Conclusions

Multidrug resistant bacteria in hospitals are on a rise around the world (including Middle East countries) and many researchers suggest methods for facing this problem [22 and 23]. One of the methods suggested was using nanoparticles.

The effect of ZnO-NPs on *E. coli* was statically significant only with high concentration used so, increasing the concentration can give good inhibition on this bacterium (Fig.3).

The method of ZnO-NPs action as antibacterial effects is not good understood, although, some studies have proposed several steps of antimicrobial reaction of this NPs, include:

1. Formation of reactive oxygen species including hydrogen peroxide (H_2O_2), that considered as strong oxidizing agent, which are harmful to cellular part of the bacteri [24-26].
2. Damage in the cell membrane and interplay of intra-cellular contents with NPs [5].

While increasing the time of irradiation of the laser with 2 mw from 5 to 10 min. couldn't give any significant difference for killing the bacterium used (Fig.4). This has acceptance with another study where He-Ne laser was used with power of 5 mw, while using Diode laser with power of 50 mw will give considerable effect on bacterium with increasing the time of irradiation [27].

Also, using the laser with ZnO-NPs don't enhance the inhibition of bacterium and the inhibition occurred in 75 ppm was related to the ZnO-NPs only that shown in figure (5), figure (6) and figure (7) when compared them with figure (3).

Other studies got more inhibition on bacterium growth by using laser with more power and irradiation time [15, 28, and 29].

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