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Investigation Of the Effect Of Laser And Nanoparticles On Different Species Of Bacteria

A Thesis

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By

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بسم الله الرحمن الرحيم

وَيُطَافُ عَلَيْهِم بِآنِيَةٍ مِّن فِضَّةٍ وَأَكْوَابٍ كَانَتْ قَوَارِيرَا (15) قَوَارِيرَ مِن فِضَّةٍ قَدَّرُوهَا تَقْدِيرًا (16) وَيُسْقَوْنَ فِيهَا كَأْسًا كَانَ مِزَاجُهَا زَنجَبِيلا(17) عَيْنًا فِيهَا تُسَمَّى سَلْسَبِيلا (18) وَيَطُوفُ عَلَيْهِمْ وِلْدَانٌ مُّخَلَّدُونَ إِذَا رَأَيْتَهُمْ حَسِبْتَهُمْ لُؤْلُؤًا مَّنثُورًا(19) وَإِذَا رَأَيْتَ ثَمَّ رَأَيْتَ نَعِيمًا وَمُلْكًا كَبِيرًا(20) عَالِيَهُمْ ثِيَابُ سُندُسٍ خُضْرٌ وَإِسْتَبْرَقٌ وَحُلُّوا أَسَاوِرَ مِن فِضَّةٍ وَسَقَاهُمْ رَبُّهُمْ شَرَابًا طَهُورًا(21)

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Dedication

I would like to
dedicate this thesis to those whom from womb to tomb ….
I am eternally indebted:

My Father..
Mother..
Brothers ..
Sisters.

My Small Family:

My Husband..
Sons..
Daughters.

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Abstract

The present study is an attempt to illustrate how the use of lasers or zinc oxide nanoparticles (ZnO NPs) and silver nanoparticles (AgNPs) each of them individually, as an antibacterial against the *Staphylococcus aureus* (*S. aureus*) which gram positive bacteria and *Escherichia coli* (*E. coli*) which gram negative as well as study the effecting result from the absorption of laser energy by these nanoparticles to kill or inhibition bacterial growth. AgNPs are prepared by biological method and by laser ablation for silver in distilled water whereas zinc oxide nanoparticles preformed by chemical method.

Transmission Electronic Microscopy (TEM), Scanning electron microscopy (SEM), Energy Dispersive X-Ray Spectroscopy (EDS) and the spectrometer region UV- Visible spectroscopy have been used to examine properties of the nanoparticles. He-Ne and diode lasers (with different irradiating powers, wavelengths and times) are used to Kill or inhibition growth of *S. aureus* and *E. coli*.

Results showed that high concentration of nanoparticles have the highest inhibitory effect on tested bacterial species (especially on *S. aureus*) whereas inhibitory action of Diode laser on tested bacterial species are more than He-Ne laser with increasing the time of irradiation until reach 20 min. This effect was due to the high power of Diode laser (with 50 mW) than He-Ne laser (with 5 mW). Also result determined increase inhibition of bacterial species (primarily *S. aureus*) by high concentration of nanoparticles with lasers at 10 min irradiations time.

In conclusions, the inhibition or killing of bacteria was increased when the concentrations of nanoparticles were amplified. In addition, sensitivity of bacteria to lasers enhance with increase time of exposure and increasing laser power.

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List of Abbreviations

Abbreviation	Key
^{0}C	Degree of celsius
Ag	Silver
AgNPs	Silver nanoparticles
C	Concentration
CW	Continuous-wave
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EDS	Energy Dispersive X-Ray Spectroscopy
ELISA	Enzyme –Linked Immunosorbent Assay
et al	et alia
g	Gram
h	hour
Hz	Hertz
ILD	injection laser diode
IT	irradiation time
Kv	Kilo volt
LD	Laser Diode
LP	laser power
LSA	laser spot area
mg	Milligram
min	Minute
mL	Milliliter

mm	**Millimeter**
mM	**Mill molar**
mw	**microwave**
ZnO	**Zinc Oxide**
nm	**nanometer**
NPs	**nanoparticles**
PDT	**Photodynamic therapy**
PLAL	**pulse laser ablation in liquid**
ppm	**parts per million**
rpm	**Revolutions per minute**
RPM	**Round per minute**
S. aureus	***Staphylococcus aureus***
SEM	**Scanning Electron Microscopy**
TCP	**Tissue Culture Plate**
TEM	**Transmission Electronic Microscopy**
U	**Unite**
UK	**United Kingdom**
USA	**United States of America**
UV	**Ultra-violet**
W	**Watt**
µg	**Microgram**
µL	**Micro liter**
µm	**micrometer**
µM	**Micro molar**
µmole	**Micromole**

Chapter One

Introduction & Previous Studies

1.1. Introduction

One of the most important causes of complications and mortality in medical centers are nosocomial infections. Disinfection of hospital surfaces is essential element for ensuring that infectious agents are not transmitted to patients. Alcohol based and chlorine-based disinfectants have unfavorable properties. Given that the antimicrobial effect of heavy metals such as silver is recognized as a viable option for eliminating bacteria, the exploration of laser and nanotechnology in this context has been described in this study [1].

The emergence of antibiotic resistance strain of both Gram-positive and Gram-negative bacteria is a major public health concern. Current advancements in laser and nanotechnology have led to the development of new techniques to synthesize nanoparticles of different size and shape and physical and chemical properties, which can be a source of development of new antibacterial agents [2,3].

Nearly most the lasers and nanoparticles have toxicological and antibacterial effect on a wide range of microorganisms[4]. The metals and metal oxides such as silver and ZnO are known to be toxic to host human cells at relatively high concentrations; but they are not expected to be toxic at very low concentrations [5]. Although the in vitro antibacterial activity and efficacy of regular zinc oxides have been investigated, little is known about the antibacterial activity of nanoparticles of silver and ZnO. Preliminary growth analysis data suggest that nanoparticles of ZnO have significantly higher antibacterial effects on *S. aureus* [6,7].

1.2. Previous Studies

Most previous studies focused on antimicrobial effect of gold and silver nanoparticles and little attentions are trended to antimicrobial action of zinc or He-Ne with 5 mW and Diode laser with 50 mW that included in this Study.

In 2010 Theivasanthi and Alagar [8] discuss the activities of silver nanoparticles synthesized by electrolysis method are more in Gram negative than Gram positive bacteria. They are investigating the changes of inner unit cell lattice constant of silver nanoparticles prepared in this method and its effects on antibacterial activities. They are noted that slight change of the lattice constant results in the enhancement of its antibacterial activities.

The effect of high-power Nd:YAG laser radiation between 50 and 300 W on *Staphylococcus aureus* 6571 (Oxford strain) was studied in 2011 by Seema and Sandeep [9], and results shows that pulse energy and exposure time are important criteria when considering inactivation of micro-organisms by laser radiation.

In 2012 Mahmood [10] found that Nanoparticles are special group of materials with unique features and extensive applications in diverse fields and use of nanoparticles of some metals is a viable solution to stop infectious diseases due to the antimicrobial properties of these nanoparticles.

In 2012 study of Al-Nori [11] observed that antibacterial properties of silver and gold nanoparticles are attributed to their total surface area, as a larger surface to volume ratio of nanoparticles provides more efficient means for enhanced antibacterial activity. Gold and silver nanoparticules was reducing gram positive and gram negative bacterial

growth. *Staphylococcus* was revealed more inhabition zone than *Streptococcus* , and *E. coli*.

Also, in 2012 the results of Rathod *et al*. [12] study show that exposure of bacterial cultures to He-Ne laser light results in a decrease in viability and the most-effective combination was 7.5mW and 90 seconds suggesting that power of laser and time of exposure plays an important role in its efficacy.

The effect of low level Diode laser radiation on *Staphylococcus aureus* with different exposure times has been studied in 2012 by Ismail *et al*. [13] who showed the effect of diode laser on the sensitivity of *S. aureus* to antibiotics discs shows slightly increase in the diameter of inhibition zone to these antibiotics at different time of exposure.

In 2014 study of McShan *et al*. [14] showed the surface of nano silver can easily be oxidized by O_2 and other molecules in the environmental and biological systems leading to the release of Ag, a known toxic ion. Therefore, nanosilver toxicity is closely related to the release of Ag.

Also, in 2014 Thangam *et al*. [15] suggest that these ZnO nano materials, which can be prepared in a simple and cost-effective manner, may be suitable for the formulation of new types of bactericidal materials.

In 2015 Wajih *et al*. [16] showed that AgNPs that synthesized by laser ablation have a great effect on *S. aureus* and *E. coli* bacteria.

In 2015 work of Chandrakanth *et al*. [17] is carried out to screen bactericidal potential of silver nanoparticles against clinically isolated multidrug resistant bacteria's. Biosynthesized silver nanoparticles were characterized by analytical techniques including as UV-Visible spectrophotometer, Field Emission Scanning Electron Microscopy,

Energy dispersive x-ray spectroscopy, Nanoparticle Tracking Analyzer analysis.

Also, in 2015 Chandrakanth *et al*. [17] showed antimicrobial effect of silver nanoparticles against *E. coli, K. pneumonia* and Methicillin resistant *Staphylococcus aureus* respectively were investigated by Agar well diffusion method.

While, in 2015 the results of Hassan [18] research showed there are noticeable changes in absorption of the bacteria after irradiation by laser with or without nanoparticles. Where absorption decreasing when the bacteria was irradiated.

In 2016 Aysa and Salman [7] detected that the small size of nano-ZnO referred which is 250 times smaller than a bacterium the might be giving it the antimicrobial ability and determine that *P. aeruginosa* isolates were completely inhibited at the concentration of 3.7 μg/ml of nano-ZnO (MIC) but no significant antibacterial activity was observed at concentrations less than 1.8 μg/ml of nano-ZnO.

1.3. The Aim of Study

The present study aims to identify the antibacterial effect of lasers on some pathogenic bacteria and also determine antibacterial effect of some nanoparticles that have the ability to interact with biological particles and microbes alone or with lasers, therefore the current research is conducted to:-

1- Study the effect of Nanoparticles (AgNPs and ZnO) on two different species of bacteria (*S. aureus* and *E.coli*).
2- Study the effect of two types of laser (He-Ne and Diode) on S. *aureus* and *E.coli.*
3- Study the mutual effect of nanoparticles and laser on *S. aureus* and *E.coli.*

Chapter Two

Theoretical Part

2.1. Introduction

This chapter includes discus and review to structure, role and types of lasers and nanoparticles as well as bacterial species that used in current research.

2.2. Laser

The word "laser" is an acronym which stands of "light amplification by stimulated emission of radiation".

The laser is one of the most important scientific and technological of the twentieth century, it has rapidly grown century, and it has rapidly grown into a major device which is now used very diverse field such as biology and medicine [19, 20].

Among their many applications, lasers are used in DNA sequencing instruments, fiber-optic and free-space optical communication; laser surgery and skin treatments; cutting and welding materials; military and law enforcement devices for marking targets and measuring range and speed; and laser lighting displays in entertainment [21].

2.2.1. Basic of Laser

A laser is very different light source than other familiar source of light, laser is a light with very high degree of coherence, both temporal and spatial, highly monochromatic and polarized, parallel with very low divergence angle.

Spatial coherence allows a laser to be focused to a tight spot, enabling applications such as laser cutting and lithography. Spatial coherence also allows a laser beam to stay narrow over great distances

(collimation), enabling applications such as laser pointers. Lasers can also have high temporal coherence, which allows them to emit light with a very narrow spectrum, i.e., they can emit a single color of light. Temporal coherence can be used to produce pulses of light as short as a femtosecond [22].

In general, the output of the lasers can emitted by continuous wave with constant of energy (CW) and by pulsing waves with manifold discrete pulses. Therefore, the two types of lasers are basically different in format, light output, and implementation. A CW laser was generated by continuously pumping energy into the active medium to perform an difference between the number of atoms raised to the excited state and the number of photons emitted. At this equipoise, CW laser output results. The interval of a CW laser pulse is approximately 0.25s [23].

In contrast, pulsed laser, deliver high-energy beams in very short pulses in the range of ms without the use of a shutter. Emission is created when the pump is changed to produce discrete laser pulses, which usually are broad and randomly shaped. Examples of pulsed laser are the copper vapor laser owing to a train of pulses at a frequency of 15000 pulses per second, which is so fast that the skin responds as it would do a continuous beam of light [24].

Q-switching lasers produce very short pulses at very high peak power. The Q-switching refers to the quality of the energy storage in the lasing medium, which is changed suddenly to produce a short, intense burst light [25].

2.2.2. Helium-Neon laser

The Helium-Neon laser was the first continuous laser. It was invented by Javan et. al. in 1961. The HeNe-Laser is the most widely used noble gas laser. Lasing can be achieved at many wavelengths (632.8 nm, 1150 nm, 3390 nm) [19].

Nowadays lasers are usually pre-adjusted using a He-Ne laser. The similarity between the manufacturing techniques of He-Ne lasers and electron valves helped in the mass production and distribution of He-Ne lasers [12].

The replacement of tubes by transistors in the sixties left a sufficiently redundant production capacity. In Germany for example, the Siemens tube factory took over this production and has produced over one million He-Ne lasers to date. He-Ne laser mechanism responsible causing bacterial death has been reported to involve the formation of singlet oxygen and free radicals [26].

It is now clear that He-Ne lasers will have to increasingly compete with laser diodes in the future. But He-Ne lasers are still unequalled as far as beam geometry and the purity of the modes are concerned. Laser diodes will have to be improved to a great extent before they pose a serious threat to He-Ne lasers [27].

2.2.3. Laser Diode

A laser diode, or LD also known as injection laser diode or ILD, is an electrically pumped semiconductor laser in which the active laser medium is formed by a p-n junction of a semiconductor diode similar to that found in a light-emitting diode [28].

Many applications of diode lasers primarily make use of the "directed energy" property of an optical beam. In this category, one might

include the laser printers, barcode readers, image scanning, illuminators, designators, optical data recording, combustion ignition, laser surgery, industrial sorting, industrial machining, and directed energy weaponry [29].

Some of these applications are well-established while others are emerging. In medicine and especially dentistry have found many new uses for diode lasers. The shrinking size and cost of the units and their increasing user friendliness makes them very attractive to clinicians for minor soft tissue procedures. Diode wavelengths range from 810 to 1,100 nm, are poorly absorbed by soft tissue, and are not used for cutting or ablation [27].

Soft tissue is not cut by the laser's beam, but is instead cut by contact with a hot charred glass tip. The laser's irradiation is highly absorbed at the distal end of the tip and heats it up to 500 °C to 900 °C. Because the tip is so hot, it can be used to sterile against microbes, cut soft-tissue and can cause hemostasis through cauterization and carbonization [30].

2.2.4. Interaction of laser light with biological tissue

The most wanted interaction was the absorption of the laser energy by the tissues. The energy that is absorbed by the intended tissue depends on the tissue properties, such as pigmentation and H_2O content, and on the wavelength of laser and emission mode [9]. The assortment of communication techniques that can be occur when applying laser to biological matter was manifold.

Definite biological material futures (optical and thermal property) and lasers parameters (exposure times, wavelengths, focal

spot sizes, applied energy, and power density and energy density) contribute to this variety [31].

Interaction of laser with biological tissues can be summarized as followed:

A- Photochemical Interaction:

The physical processes involved in the interaction of a laser rays and a tissue were divided into three parts [32]:

(1) Laser energy absorption.

(2) Transformation of the laser energy into chemical energy and/or into heat, and diffusion of heat away from the exposed area.

(3) Eventually, chemical reaction and/or phase changing (in general, vaporization).

B- Biostimulation

Biostimulation also attributed to photochemical interactions, which believed to occur at low irradiations, where the exposing of the cells at certain wavelengths can activate some of the native components and in this way specific biochemical reactions in addition whole cellular metabolic rate can be changed. Biostimulation can be used in the healing of wound and anti-inflammatory properties by red or near infrared light sources like He- Ne laser or Diode laser [31].

C- Photothermal interaction

The thermal effect of laser on biological tissue was complicated process that result from three phenomena; conversion of laser light to heat, transfer of heat, and finally tissue reaction, which is related to the temperature degree and heat time. Depending on duration and peak

value of tissue temperature achieved, different effects such as coagulation, vaporization, and melting can be distinguished [9].

D- Photoablation

Photoablation involved in spontaneous etching which occurs upon the absorption at the material surface by a pulse of laser light. The advantage of using UV light resides, in the fact that, the photoablation is strictly confined to the volume that absorbs the laser energy [33, 34].

2.3. Nanoparticles

2.3.1.Definition

Nanoparticles are particles between 1 and 100 nanometers in size. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. The term "nanoparticle" is not usually applied to individual molecules; it usually refers to inorganic materials. The reason for the synonymous definition of nanoparticles and ultrafine particles is that, during the 1970s and 80s, when the first thorough fundamental studies with "nanoparticles" were underway in the USA and Japan, they were called "ultrafine particles"[35].

However, during the 1990s before the National Nanotechnology Initiative was launched in the USA, the new name, "nanoparticle," had become more common. Nanoparticles can exhibit size-related properties significantly different from those of either fine particles or bulk materials [36].

2.3.2. Properties

Nanoparticles are of great scientific interest as they are, in effect, a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nano-scale size-dependent properties are often observed. Thus, the properties of materials change as their size approaches the nanoscale and as the percentage of the surface in relation to the percentage of the volume of a material becomes significant. For bulk materials larger than one micrometer (or micron), the percentage of the surface is insignificant in relation to the volume in the bulk of the material. Nanoparticles often possess unexpected optical properties as they are small enough to confine their electrons and produce quantum effects [37].

Other size-dependent property changes include quantum confinement in semiconductor particles, surface plasmon resonance in some metal particles and super paramagnetism in magnetic materials. What would appear ironic is that the changes in physical properties are not always desirable. Ferromagnetic materials smaller than 10 nm can switch their magnetisation direction using room temperature thermal energy, thus making them unsuitable for memory storage [38].

Suspensions of nanoparticles are possible since the interaction of the particle surface with the solvent is strong enough to overcome density differences, which otherwise usually result in a material either sinking or floating in a liquid. The high surface area to volume ratio of nanoparticles provides a tremendous driving force for diffusion, especially at elevated temperatures. Sintering can take place at lower temperatures, over shorter time scales than for larger particles [39].

In theory, this does not affect the density of the final product, though flow difficulties and the tendency of nanoparticles to agglomerate complicates matters. Moreover, nanoparticles have been found to impart some extra properties to various day to day products. For example, the presence of titanium dioxide nanoparticles imparts what we call the self-cleaning effect, and, the size being nano-range, the particles cannot be observed. Zinc oxide particles have been found to have superior UV blocking properties compared to its bulk substitute. This is one of the reasons why it is often used in the preparation of sunscreen lotions, is completely photostable and toxic [40].

2.3.3. Synthesis of Nanoparticles

Nanoparticles can be synthesized physically or chemically or biologically. Many adverse effects have been associated with chemical synthesis methods due to the presence of some toxic chemical absorbed on the surface. Eco friendly alternatives to Chemical and physical methods are Biological ways of nanoparticles synthesis using microorganisms, enzymes, fungus, and plants or plant extracts [41,42].

The development of these eco friendly methods for the synthesis of nanoparticles is evolving into an important branch of nanotechnology especially Zinc and silver nanoparticles, which have many applications [40,43].

Biosynthesis of Nanoparticles by microorganisms is a green and eco-friendly technology. Diverse microorganisms, both prokaryotes and eukaryotes are used for synthesis of metallic nanoparticles viz. silver, gold, platinum, zirconium, palladium, iron, cadmium and metal oxides such as titanium oxide, zinc oxide, etc. These microorganisms include bacteria, actinomycetes, fungi and algae. The synthesis of

nanoparticles may be intracellular or extracellular according to the location of nanoparticles [44,45].

Recently, there were many methods for synthesis of nanoparticles, some of these modern methods are [37]:

1- Milling method: a mechanical method used to produce powder nanomaterial, where the material was put under very high energy and milling it with iron balls.
2- Etching method: used to produce nano Silicon molecules, by using chemical or electrochemical methods.
3- Sputtering method: used to produce thin films, where the material put under very low pressure in the evacuated container with cooled base in a magnetic field.
4- Laser ablation

Laser ablation (LA) is process in which a laser beam is focused on a sample surface to remove material from the irradiated zone. Laser ablation has been considered and used for many technical applications, including the production of nanomaterials, deposition of thin metallic, dielectric films and fabrication of superconducting materials. Recently, laser ablation has gained popularity as a manufacturing for solar cell.

AgNPs synthesis by pulse laser ablation in liquid (PLAL) permits the prepration of stable Ag colloids in pure solvents without capping or stabilizing agents, producing AgNPs more stable for biomedical applications [38].

Two types of nanoparticles were used in this study which are:

A- Silver nanoparticles

Silver nanoparticles are nanoparticles of silver of between 1 nm and 100 nm in size. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms. Numerous shapes of nanoparticles can be constructed depending on the application at hand. Commonly used are spherical silver nanoparticles but diamond, octagonal and thin sheets are also popular [46] .Silver nanoparticles have proved to be most effective because of it's good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms [47]. They are undoubtedly the most widely used nanomaterials among all, thereby being used as antimicrobial agents, in textile industries [48].

B- Zinc Oxide Nanoparticles

Zinc oxide nanoparticles have become famous among researchers due to its use in various applications like gas sensors, chemical sensors, biosensors, superconductors, photo catalyst, optoelectronic devices and cosmetics [49]. ZnO is a wide band gap semiconductor having high optical transparency and luminescence in visible and near ultraviolet range of spectrum. Therefore, it is usually used in light emitting diodes and solar cells. ZnO nano particles are having high exciton binding. Moreover Zinc Oxide is environmental friendly and ease to synthesize [50]. Many techniques are being used to synthesize ZnO nanoparticles Viz. precipitation method, spray pyrolysis method, micro emulsion method, hydrothermal method and Sol gel method. The sol-gel method for synthesis of ZnO nanoparticles was chosen as it is simplest method, consumes less power and can be carried out in robust atmosphere [51].

ZnO NPs are being investigated as associates of antibacterial agents in each microscale and nanoscale formulation. The ZnO NPs are widely wont to treat a range of different skin conditions and have anticancer properties. In addition, ZnO NPs have emerged as a suitable tool in drug delivery and sensing horizon [51].

2.3.4. Silver Nanoparticles Test (Lattice Constant)

Cubic structure of AgNPs prepared by method of electrolysis was shown from computed XRD. Nano size was deduced by using Debey-Scherrer equation [8]:

$$D = \frac{0.9\lambda}{\beta cos\theta} \quad \text{..... (2-1)}$$

Where

β : FWHM (full width at half maximum).

λ: wave length of X-Ray (0.1541 nm).

D: particle diameter.

θ: the diffraction angle

D: particle diameter size.

2.4. Bacteria

Bacteria are microscopic single-celled organisms that thrive in diverse environments. They can live within soil, in the ocean and inside the human gut. Bacteria are prokaryotes and entirely consists of a single cell with a simple internal structure [52].

The gram stain is a test used to identify bacteria by the composition of their cell walls (figure 2-1), It is named for Hans Christian Gram, who

developed the technique in 1884. Bacteria are first stained with a purple dye called crystal violet, which specifically binds to peptidoglycan, a complex structure of amino acids and sugars found in the cell wall. This is followed by a series of steps that ultimately remove any unbound or loosely bound crystal violet. Then the cells are stained with a second red-colored dye called safranin. Gram-positive bacteria stain purple because their cell walls are rich in peptidoglycan [53, 54]. On the other hand, gram-negative bacteria whose cells walls have two layers take on a red coloring. The outer layer of lipids does not bind strongly to crystal violet and the dye is easily washed away during the staining process. For example, *S. aureus*, is a gram-positive bacterium, while *E. coli* is gram-negative bacteria [55]. These bacteria used in current study because they are resist to many of antibiotics and cause serious infections.

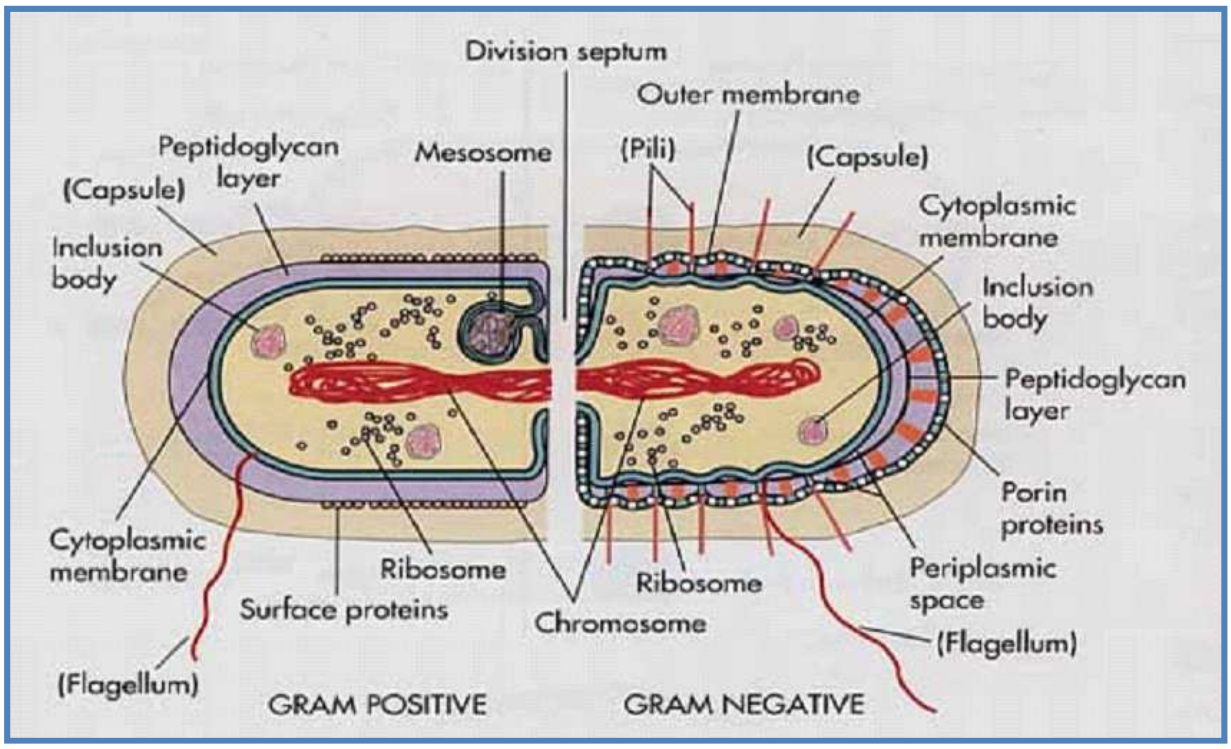


Figure (2-1): Different between gram positive and gram negative bacteria [56].

2.4.1. *Escherichia coli (E.coli)*

Escherichia coli is a member of the family *Enterobacteriaceae*, a gram-negative, rod shaped and facultative anaerobic bacterium (figure 2-2) [57]. *E. coli* bacteria were discovered in the human colon in 1885 by German bacteriologist Theodor Escherich. Escherich also showed that certain strains of the bacterium were responsible for infant diarrhea and gastroenteritis and that an important public health discovery. Although *E. coli* bacteria were initially called Bacterium coli, the name was later changed to *Escherichia coli* to honor its discoverer [58].

Figure (2-2): Plate of *E. coli*.

It is a highly versatile bacterial species comprised of both harmless commensal strains and different pathogenic variants with the ability to cause either intestinal or extraintestinal diseases [59]. Consequently, *E. coli* strains are broadly classified into three major groups of commensal *E. coli*, intestinal pathogenic *E. coli* and extraintestinal pathogenic *E. coli* [56]. The non pathogenic strains of *E. coli* referred to as commensal strains are harmless and are useful, not only in digesting and breaking down food, but also in protecting against harmful organisms which may be introduced into the gastrointestinal tract through food and water [60].

2.4.2. *Staphylococcus aureus (S. aureus)*

S. aureus is a Gram-positive, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus (figure 2-3). The *Staphylococcus* genus is subdivided into 32 species and subspecies. *S. aureus* produces staphylococcal enterotoxin and is responsible for almost all staphylococcal food poisoning [61]. *S. aureus* is a facultative anaerobe so can grow under both aerobic and anaerobic conditions. However, growth occurs at a much slower rate under anaerobic conditions [62].

Currently, greater than 60% of *S. aureus* isolates are resistant to Methicillin and some strains have developed which are resistance to more than 20 different antimicrobial agents [63]. Infections caused by multiple-antibiotic-resistant *S. aureus* strains (e.g., Methicillin-Resistant *S. aureus* (MRSA) are particularly difficult to treat, and MRSA infections are often associated with higher mortality and increased healthcare costs compared with Methicillin-sensitive strain infections [64].

Figure (2-3): plate of *S. aureus*.

Chapter Three

Materials and Methods

3.1. Introduction

This chapter includes the description of the research devices, materials, tools, samples collections and practical steps for synthesis and application of nanoparticles and laser (in different power and exposure times) on the tested Bacterial species.

3.1.1. The devices

The devices that used in the current study are listed in table (3-1).

Table (3-1): Represent the used devices with their remarks.

The device name	Made
Autoclave	USA
Automatic water still	Korea
Digital laser power measurement	Germany
Diode laser (50 mW)	Germany
Enzyme –Linked Immunosorbent Assay (ELISA)	USA
Electronic weighing balance	Germany
Helium- Neon laser (5 mW)	Germany
Hood	Korea
Hot plate	Germany
Incubater	Germany
Nd-YAG 1064 (20 W)	Germany
Refrigerator (for keeping the bacterial cultures)	Korea
Shaking incubator	Germany
Transmission Electronic Microscopy (TEM)	Germany
UV-Visible Spectrophotometer	Germany
Water bath	Germany

3.1.2. Materials

The physicals, chemicals and biological materials that are used in this study are listed in table (3-2).

Table (3-2): Represent the used physicals, chemicals and biological materials with their remarks.

Materials	Made
Bacterial species (*E. coli* and *S. aureus*)	Central health laboratory- Al Najaf city
Dimethyl sulfoxide (DMSO)	Germany
Distal water	From microbiology Laboratory for postgraduates research - College of medicine - University of Al-Qadisiya.
Muller Hinton agar	India
Nutrient broth	India
Silver nanoparticles-1	prepared (by laser ablation) in the Nanotechnology Lab.- University of Kufa - College of Engineering.
Silver nanoparticles-2	prepared (by the biosynthesis method) in the Microbiology Laboratory- for postgraduates research - College of medicine - University of Al-Qadisiyah.
ZnO nanoparticles	prepared (by the Chemical method) in the Clinical and Laboratory Sciences Dept. College of Pharmacy - University of Babylon.

3.1.3. Tools

The tools and equipments that used in the current study are listed in table (3-3).

Table (3-3): Represent the used tools and equipments with their remarks.

Tool name	Remarks
Plate	Sturdy (Taiwan)
Beaker	AMSCO (Germany)
Glass flasks	Himedia (India)
Bunzen burner	Broche (Malaysia)
Micropipet	Hettich (Germany)
Loop	AMSCO (Germany)
Stands and clamps	GFL (Germany)
Gloves	BioMérieux / USA
Cotton	Sanyo (Japan)
Tape	GFL (Germany)
Filter paper	Sterilin Ltd. / UK
Tissue culture plate (TCP)	AMSCO (Germany)
Tubes	China
Eppendrof tubes	Shinsaeng (South Korea)
Swap	Consort (Belgium)
Respiratoy mask (USA)	Heidolph (Germany)
Washing bottles	China
Test tube rack	Jarad (Syria)
Filter paper	Lab Tech (korea)
Funnel	Sterilin Ltd. / UK
Graduated cylinders	Eppendorf (germany)
Glasses	Sterilin Ltd. / UK

3.2. Preparation of bacterial samples and culture media

This section includes the description of preparation of the Nutrient broth, Muller Hinton agar and preparation of bacterial samples

3.2.1. Preparation of Nutrient Broth

Suspend 13g of Nutrient Broth that measured by sensitive electronic balance (shown in the figure 3-1) was dissolved in 1000 ml distal water, the mixture was mixed and shaked and placed on hot plate heater to boil to dissolve the medium completely, then the mixture was sterilized by Autoclaving (figure 3-2) at 121 °C for 15 min. After that, waiting mixture for cooling and placed in small tubes, then keep them in the refrigerator (the method for preparation was found in the cover of the agar container).

Figure (3-1): Represent image sensitive electronic balance.

Figure (3-2): Represent image for Autoclave.

3.2.2. Preparation of Bacterial Samples

The bacterial samples were *E. coli* and *S. aureus* that collected from the surgical tools in the operation rooms of the Al- Najaf teaching hospital. The collection was done by swap and then the bacteria was cultured and diagnosed in the Central Health Laboratory in Al Najaf city. After that the *E. coli* (gram negative) and *S. aureus* (gram positive) were sub cultured in tubes of Nutrient Broth agar by sterilized loop in the Hood that contain benzene burner to get sterilized environment , then placed in the incubator for 24 hrs in 37°C for growth.

3.2.3. Preparation of Muller Hinton agar

Suspend 19g of Muller Hinton agar was dissolved in 500 ml distilled water, the mixture was mixing and shaking and placed on hot plate heater to boiling to dissolve the medium completely, then the mixture was sterilize by Autoclaving at 121 °C for 15 min. After that, waiting mixture for cooling (about 50°C) and placed in plates and after

solidification, kept them in the refrigerator. The bacteria were sub cultured in plates of Muller Hinton agar by swap then placed in the incubator for 30 min in 37°C for growth (the method for preparation was found in the cover of the agar container).

3.3. Nanoparticles preparation

This section includes the description of preparation for Silver nanoparticles (by laser ablation and biological methods) and ZnO nanoparticles (by chemical method).

3.3.1. Silver Nanoparticles Preparation

A- Laser Ablation

The preparing was done by laser ablation where Nd-YAG laser (1064 nm wave length) was applied with red pulse light and max energy 1000 mj for each pulse. Pulse period 7 nanosecond with average reputation 6 Hz and active beam diameter 5 mm, used for laser ablation. Lens used with focal length 15.3 cm (figure 3-3).

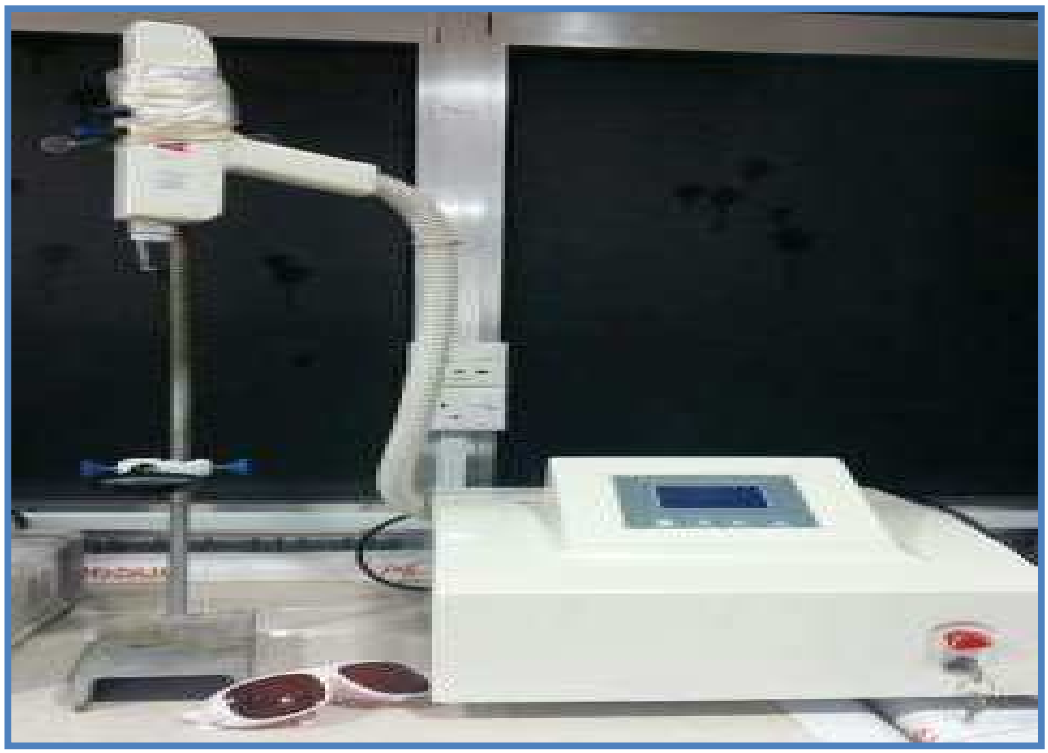


Figure (3-3): Represent image for Nd-YAG laser.

Colloidal nano silver that result has a particle size (20 to 40) nm (average = 30 nm) with concentration 250 ppm. The prepared concentration in this study were (5, 10, 15, 50) ppm that equal to mg/L. The concentrations were prepared by the dilution equation [37]:-

$$C_1 V_1 = C_2 V_2 \quad \text{……..(3-1)}$$

Where:

C_1: the original concentration

V_1: the volume that need to dilute it.

C_2: The required concentration.

V_2: The final volume that result from the preparation.

The same equation was used for all the dilution made in this study.

B- Biosynthesis Method:

***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 [65].

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 15 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) [65].

3.3.2. Nano Zinc Oxide preparation

Dissolve acetate zinc in a mixture of methanol and mono ethanol secretary at room temperature and then mixed with a magnetic mixer for one hour until a homogenous solution occur, left for 24 hours and then the solution is heated for 3 hours at 200 °C, the black material precipitate calcined at 500 °C and then collected a white powder (nano zinc oxide).

Preparation of Nano ZnO Concentration

Nano zinc oxide was weighed as 10 mg then dissolved in 10 ml dimethyl sulfoxide (DMSO) yielding stock solutions of 1 mg/ml after that 1 ml of this solution was diluted to 10 ml with DMSO again giving a solution of 100 μg/ml concentration, then from this solution, the required concentration which include: 50, 75, 100 μg/ml had been prepared [7].

3.4. Test Devices

This section includes the description of the instruments used for test of the nanoparticles which are:

3.4.1. UV-Visible Spectrophotometer

This device covers a wide area from the electromagnetic spectrum (from the ultraviolet region to the infrared region). Range from 190 to 1100 nm, Made in Germany by analytik jena company as seen in figure (3-4).

The spectrum measurements for colloidal silver nanoparticles (NPs) were done with room temperature in quartz cell in the biology laboratory- College of medicine, University of Al-Qadisiyah.

Figure (3-4): Represent a picture of UV-Visible Spectrophotometer.

3.4.2. Transmission Electronic Microscopy (TEM).

NPs samples were recognized by TEM (Model: LEO 912 AB, made in Germany), the samples were tested in the central laboratory, Ferdossy university, Iran.

Test time is consumed 2 hrs for each sample, and the preparation of the sample consume 1hr, where one droplet from the colloidal NPs placed on the copper grid covered with non crystallized carbonic film, the droplet was dried with infrared lamp (Philips-100 W), then the copper grid was tested. The images were got with (120 kV) acceleration voltage with max magnification (25000x-63000x) as shown in figure (3-5).

Figure (3-5): Picture for Transmission Electronic Microscopy.

3.4.3. Scanning Electron Microscopy (SEM).

Biosynthesis NPs samples were recognized by SEM Inspect S50, FBI company, made in Netherlands (figure 3-6). The samples were tested in the research laboratory, University of Al-Kufa, Iraq.

Figure (3-6): Picture for Scanning Electron Microscopy.

3.5. Application Nanoparticles on Bacterial Species Cultured on Muller Hinton Agar.

This section includes the procedure for applying the nanoparticles (AgNPs and nano ZnO) on bacteria that cultured in the Muller Hinton agar which considered as solid plates**.**

3.5.1. Application Silver nanoparticles on Bacterial Species

A- AgNPs Prepared by Laser Ablation

1- The bacteria (*E. coli* and *S. aureus*) those growths in Muller Hinton agar were taken then holes were made with 5 mm in diameter for each one.

2- Placed 0.2 ml of colloidal AgNPs in 3 holes.

3- Placed a distill water in other hole as control.

4- Put the plates in the incubator for 24 hrs. in temperature of 37°C.

5- The steps above were done according to different concentration (5, 10, 15, 50, and 250) ppm that prepared previously.

6- The inhibition zone will be determined for the holes in each plate.

B- AgNPs Prepared by Biosynthesis Method

The same procedure above was repeated by using AgNPs prepared by the biosynthesis method. The difference was in the concentration used (36.2, 18.1, 9.1) ppm.

3.5.2. Application Nano ZnO on Bacterial Species

1- The bacteria that growth in Muller Hinton agar were taken and making holes , the diameter for each hole was 5 mm

2- Placed 0.2 ml of colloidal nano ZnO in 5 holes.

3- Placed DSMO in other hole as control.

4- Put the plates in the incubator for 24 hrs. in temperature of 37ċ.

5- The steps above were done according to different concentration 50, 75, 100 μg/ml that prepared previously.

6- The inhibition zone will be determined for the holes in each plate.

3.6. Application Nanoparticles on Bacterial Species Cultured on Nutrient Broth

This section includes the procedure for applying the nanoparticles (AgNPs and nano ZnO) on bacteria that cultured in the Nutrient broth.

3.6.1. Application Silver nanoparticles on Bacterial Species

AgNPs that used was gained by the biological method in the following steps:

1- Making sub culture in the small tubes of nutrient broth that have been prepared previously.

2- Placed 0.2 ml from the colloidal AgNPs in each tube according to the prepared concentration (36.2, 18.1, 9.1) ppm.

3- Put the tubes in the incubator for 24 hrs. in temperature of 37ċ**.**

4- Observe the amount of killing, this will be considered as a second control for laser irradiation in the ELISA test in the Research Laboratory- College of Medicine- University of Al-Qadisiyah (figure 3-7).

Figure (3-7): Represent a picture for the ELISA device with computer.

3.6.2. Application Nano ZnO on Bacterial Species

Nano ZnO was used as following procedure:

1- Making sub culture in the small tubes of nutrient broth that have been prepared previously.

2- Placed 0.2 ml from the colloidal ZnO NPs in each tubes according to the prepared concentration 50, 75, 100 and 1000 μg/ml.

3- Put the tubes in the incubator for 24 hrs. in temperature of 37°C**.**

4- Observe the amount of killing, (this will be considered as a second control for laser irradiation in the ELISA test).

3.7. Application Laser Irradiations on Bacterial Species

This section includes the description the procedure for applying the Helium Neon laser (with 5 mW) and diode laser (with 50 mW) on the bacterial samples.

3.7.1. Application Helium Neon Laser (5 mW) on Bacterial Species

This laser with 632.8 nm wave length and red color, from the Optics Physics laboratory- Dept. of Physics- College of Education- University of Al-Qadisiyah, as seen in figure (3-8). As in the following steps:

1- Take tube that contains *E. coli* (that cultured in the nutrient broth). This will be considered as control.

2- Place the laser device in vertical position to control the direction of beam to be applied directly to the samples.

3- Take other tubes that contain the same type of bacteria and irradiates them by laser with different exposure times (5, 10, 20) min.

4- Take 0.2 ml from each tube by micropipette and put them in the TCP for later test in ELISA device.

5- The same steps above were repeated for *S. aureus*.

6- Laser dose was estimated in each irradiation, where the production power was calculated by using digital laser power measurement device (figure 3-9).

Figure (3-8): Represent a picture for He-Ne laser with 5 mW.

Figure (3-9): Represent a picture for digital laser power measurement device.

Also, the diameter of the laser spot was measured (1 mm), and then the laser dose was measured according to the following formula [37]:

$$\textbf{Dose= LP (w) X IT (sec.) / LSA (cm}^2\textbf{)} \quad \text{……..(3-2)}$$

Where:

LP: laser power

IT: irradiation time

LSA: laser spot area

So, the range of the doses used in this irradiation were (76.43 to 458.59) J/cm^2.

Laser dose was calculated according to the (3-2) formula, and the range for the laser dose (from 191.1 to 1146.5) J/cm^2.Where the spot diameter measured was 1 mm.

3.7.2. Application Diode laser (50 mW) on Bacterial Species

This laser was used with 650 nm and red color, from the Optics Physics laboratory- Dept. of Physics- College of Education- University of Al-Qadisiyah, as shown in figure (3-10).

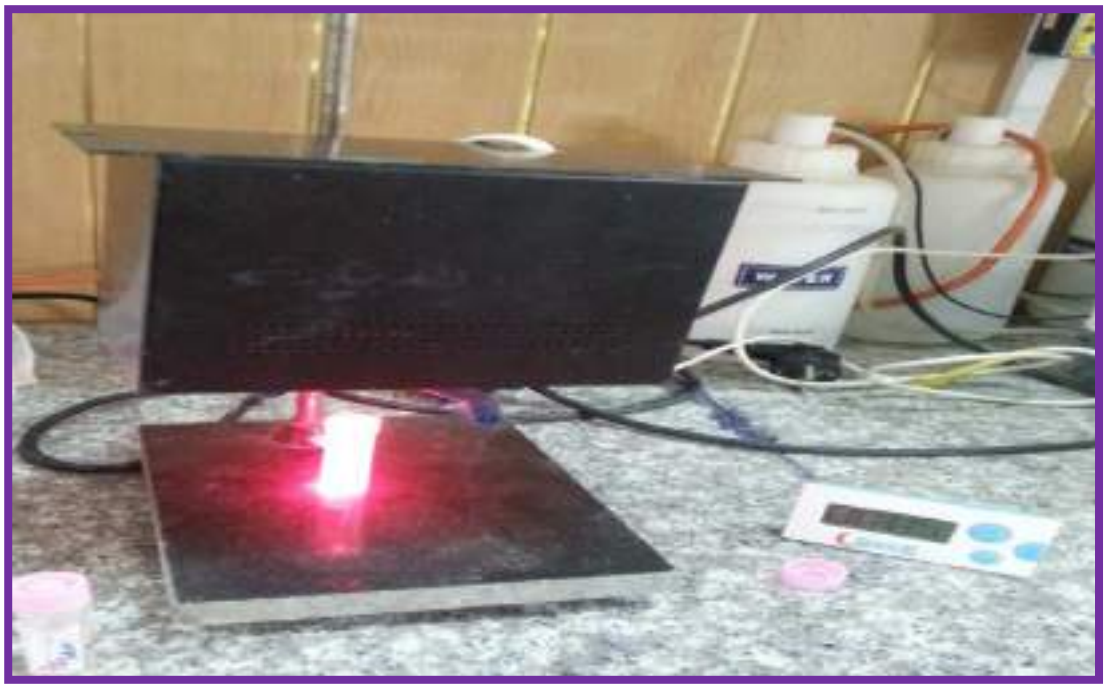


Figure (3-10): Represent a picture for Diode laser with 50 mW.

The same procedure was repeated as for Helium Neon laser (5 mW), but with exposure time (1, 3, 5, 10, 20) min. Also, laser Dose was measured according to the (3-2) formula, and the range for the laser dose (from 95.5 to 2866.2 J/cm^2).Where the spot diameter measured was 2 mm.

3.8. Application Nanoparticles with Laser Irradiation on Bacterial Species

This section includes the procedure for applying He-Ne (5 mW) and diode laser on bacteria with AgNPs or with nano ZnO.

3.8.1. Application AgNPs with Laser on Bacterial Species

Each type of laser was directed vertically toward the bacteria with colloidal AgNPs.

A- Application of AgNPs with He-Ne laser on Bacterial Species

AgNPs that used was result from the biological method and as in the following steps:

1. Take 0.2 ml from *E. coli* bacteria (cultured previously in tubes of nutrient broth agar) by micro pipette and put it in the TCP, and considered as first control.
2. Take 0.2 ml of *E. coli* bacteria that cultured previously in tubes of nutrient broth agar with AgNPs and put them in TCP and considered as a second control.
3. Place the laser device in vertical position to control the direction of beam to be applied directly to the samples.
4. Take other tubes that contain *E. coli* bacteria with AgNPs and irradiate them with He-Ne laser (5mW) separately and exposure times (5 and 10) min. using the prepared concentration (36.2, 18.1, 9.1) ppm.
5. Take 0.2 ml from each tube and put them in TCP
6. Observe the amount of killing (ELISA test).
7. Made the same steps for *S. aureus* bacteria.
8. The same procedure was repeated when irradiates with He-Ne laser with 5 mW.

B- Application AgNPs With Diode Laser on Bacterial Species

AgNPs that used was result from the biological method and the below steps was followed:

1- Take 0.2 ml from *E. coli* bacteria (cultured previously in tubes of nutrient broth agar) by micro pipette and put it in the TCP, and considered as first control.
2- Take 0.2 ml of *E. coli* bacteria that cultured previously in tubes of nutrient broth agar with AgNPs and put them in TCP and considered as a second control.
3- Place the laser device in vertical position to control the direction of beam to be applied directly to the samples.
4- Take other tubes that contain *E. coli* bacteria with AgNPs and irradiate them separately with exposure times (1, 3, 5, 10, 20) min. using the prepared concentration (36.2, 18.1, 9.1) ppm.
5- Take 0.2 ml from each tube and put them in TCP
6- Observe the amount of killing (ELISA test).
7- Made the same steps for *S. aureus* bacteria.

3.8.2. Application ZnO NPs with Laser on Bacterial Species

1- Take 0.2 ml from *E. coli* bacteria (cultured previously in tubes of nutrient broth) by micro pipette and put it in the TCP, and considered as first control.
2- Take 0.2 ml of *E. coli* bacteria that cultured previously in tubes of nutrient broth with ZnO NPs and put them in TCP and considered as a second control.
3- Take other tubes that contain *E. coli* bacteria with ZnO NPs and irradiate them with He-Ne laser (5mW) separately and exposure times (5 and 10) min. with the prepared concentration of ZnO NPs 50, 75, 100 and 1000 μg/ml.
4- Take 0.2 ml from each tube and put them in TCP
5- Observe the amount of killing (ELISA test).
6- Made the same steps for *S. aureus* bacteria.

7- The same procedure was repeated when irradiates with Diode laser with 50 mW, but with exposure times (1, 3, 5, 10, 20) min.

3.9. Research Safety

Safety steps must be followed for protection from the hazards of bacteria, nanoparticles and laser as following:

1- Gloves must be used to protect the hands from bacteria and nanoparticles.

2- Special respiratory mask must be used to protect from nanoparticles and bacteria (figure 3-11). Hood that contain benzene burner to get sterilized environment (figure 3-12).

3- Protective glass must be worn in laser areas to protect the eyes from reflected or scattered beam during irradiation.

4- Washing hands after working with bacteria and nanoparticles with sterilized materials like disinfected soap.

Figure (3-11): Represent a picture for special mask.

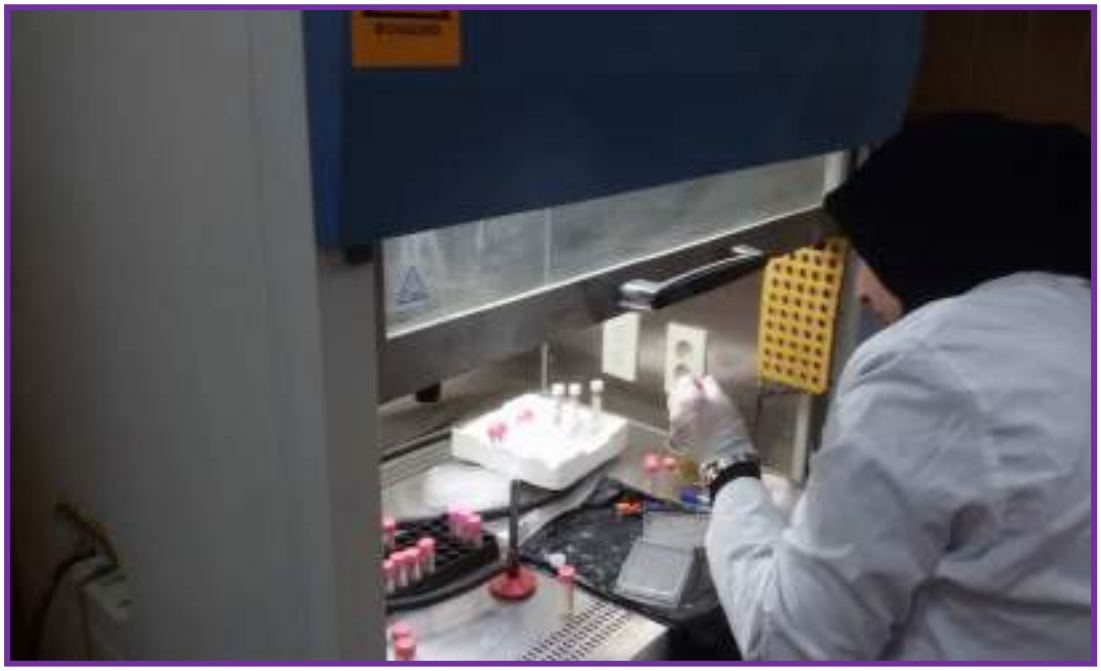


Figure (3-12): Represent image for working in Hood with benzene burner.

Chapter Four

Results and Discussion

4.4. Effect of Nanoparticles on Bacterial Species Cultured on Nutrient Broth

This section includes the description of the result of effects for nanoparicles (AgNPs and ZnO NPs) on *E. coli* and *S. aureus* that cultured in Nutrient broth (Which is liquid media).

4.4.1. Effect of Silver Nanoparticles on Bacterial Species

There are two types of test to observe the effect of AgNPs on bacteria which are:

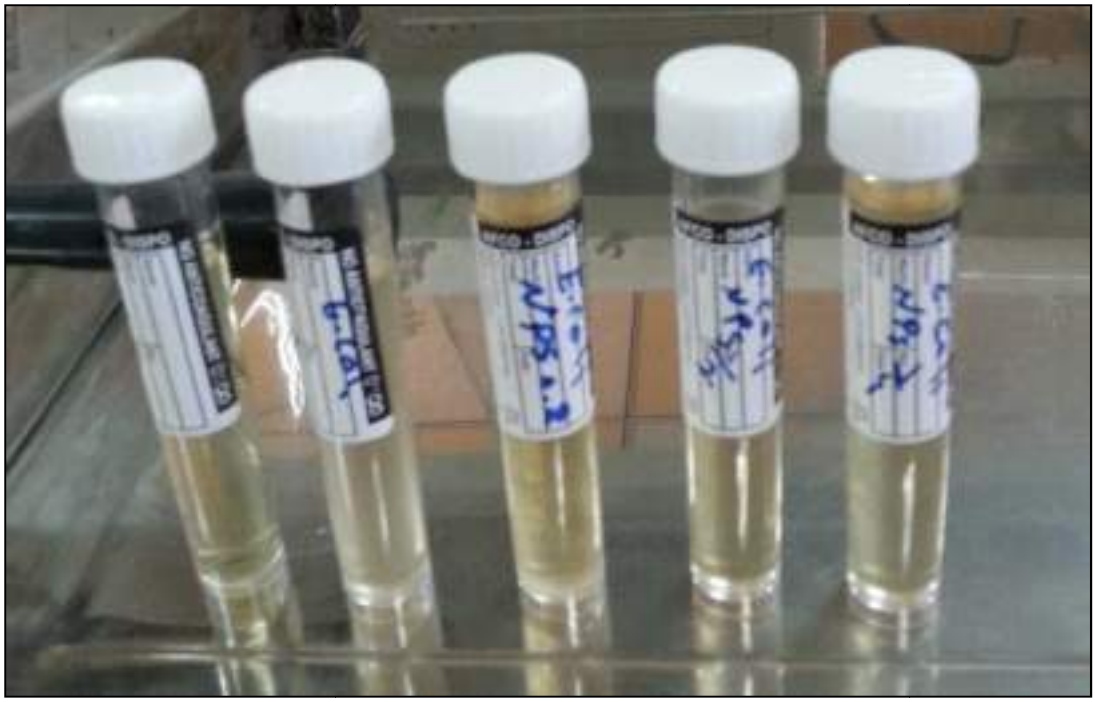
1. Direct vision to the tubes that contain bacteria and AgNPs in different concentration, as seen in the figure (4-13 a) for *E. coli* and figure (4-14 a) for *S. aureus*.

Test by ELISA device, seen in the figure (4-13 b) for *E. coli* and figure (4-14 b) for *S. aureus*. Result showed that lower concentration of AgNPs have highest effect on bacteria (especially on *S. aureus*).

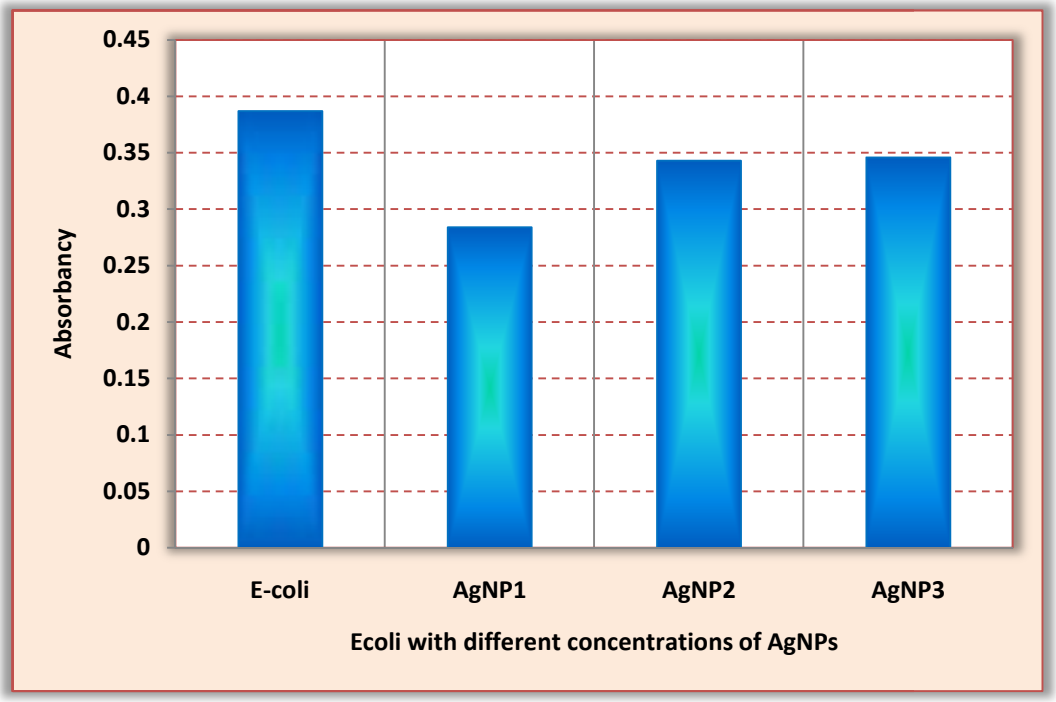
4.4.2. Effect of ZnO Nanoparticles on Bacterial Species

There are two types of test to observe the effect of ZnO NPs on bacteria which are:

1. Direct vision to the tubes that contain bacteria and ZnO NPs in different concentration, as seen in the figure (4-15 a) for *E. coli* and figure (4-16 a) for *S. aureus*.
2. Test by ELISA device, that seen in the figure (4-15 b) for *E. coli* and figure (4-16 b) for *S. aureus*. Result showed that lower concentration of ZnO NPs have highest effect on bacteria (especially on *S. aureus*).



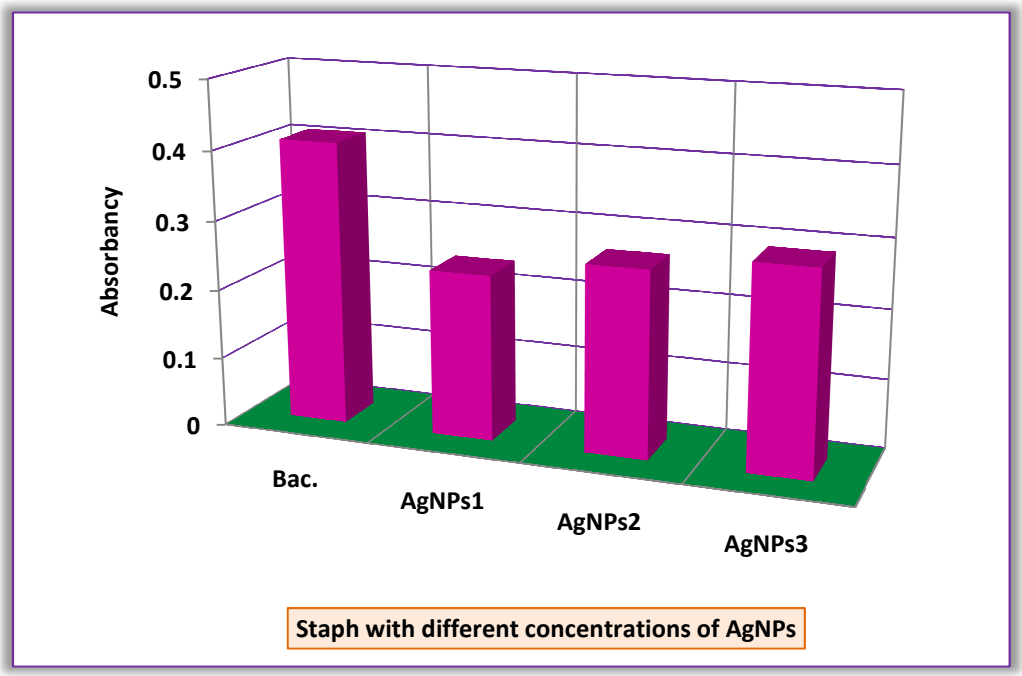
a



b

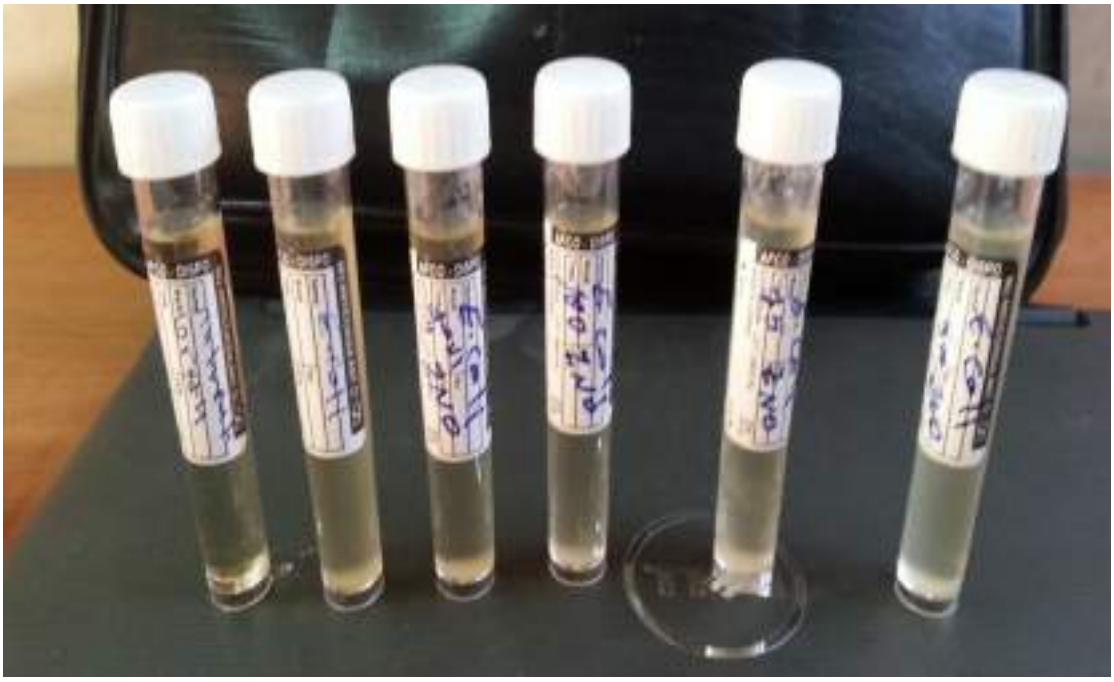
Figure (4-13): Represents image (a) and drawing (b) for AgNPs with *E.coli* in Nutrient broth.

a

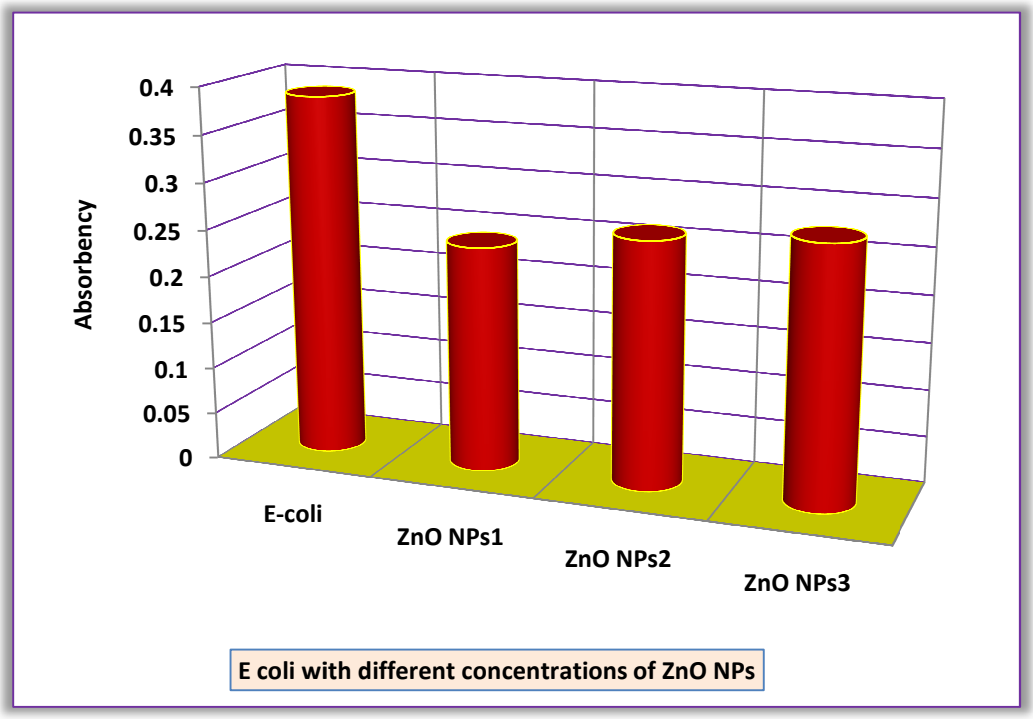


b

Figure (4-14): Represents image (a) and drawing (b) for AgNPs with *S. aureus* in Nutrient broth.

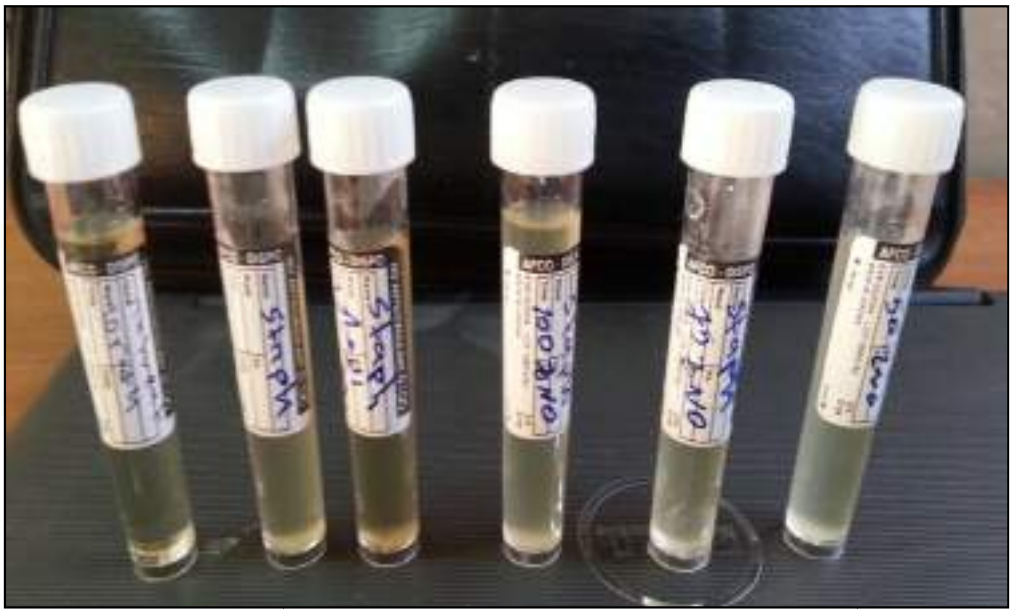


a

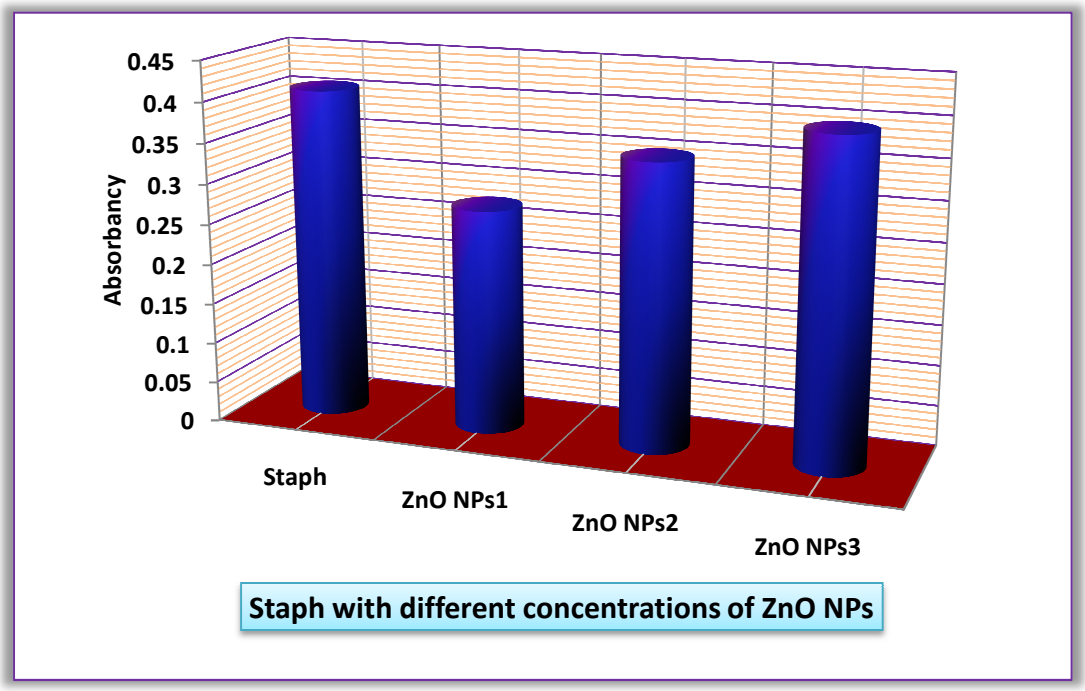


b

Figure (4-15): a- Represents image for *E.coli* and ZnO in tubes (with different concentrations), b- ELISA test for *E. coli* with ZnO NPs in different concentrations.



a



b

Figure (4-16): a- Represents image for *S. aureus* and ZnO in tubes (with different concentrations), b- ELISA test for *S. aureus* with ZnO NPs in different concentrations.

Amro *et al* (2000) 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 [75].

Antibacterial effect of ZnO NPs may be due to ability of nano ZnO to disrupt the bacterial cell membrane integrity (the particles interact with the building elements of the outer membrane and might cause structural changes), reduce cell surface hydrophobicity and down-regulate the transcription of oxidative stress-resistance genes in bacteria, then degradation and finally cell death [72].

Theivasanthi and Alagar (2010) showed that the toxicity of silver nanospheres and nano ZnO is higher than that of gold nanospheres and bacteria not able to develop immunity to nano ZnO or silver as they often do with antibiotics [8].

In this study antimicrobial action of used nanoparticles enhance with increase their concentration and this agree with other studies [7,8]. Current study detected that *S. aureus* is more sensitive to nanoparticles than *E. coli* this may be related to present outer membrane in *E. coli* and absent in *S. aureus*.

4.5. Effect of Laser Irradiations on tested Bacterial Species

This section includes the effect of two types of laser which are He-Ne (5 mW) and Diode (50 mW) on *E. coli* and *S. aureus*.

4.5.1. He- Ne laser (5 mW)

The effect of He-Ne laser with 5 mW in different irradiation times on *E. coli* and *S. aureus* can be seen in the figure (4-17) and figure (4-18)

respectively that showed lower absorbance are appeared at 15 min and 20 min Irradiations time

4.5.2. Diode laser (50 mW)

The effect of Diode laser with 50 mW in different irradiation times on *E. coli* and *S. aureus* can be seen in the figure (4-19) and figure (4-20) respectively that showed lower absorbance are appeared at 20 min irradiations time.

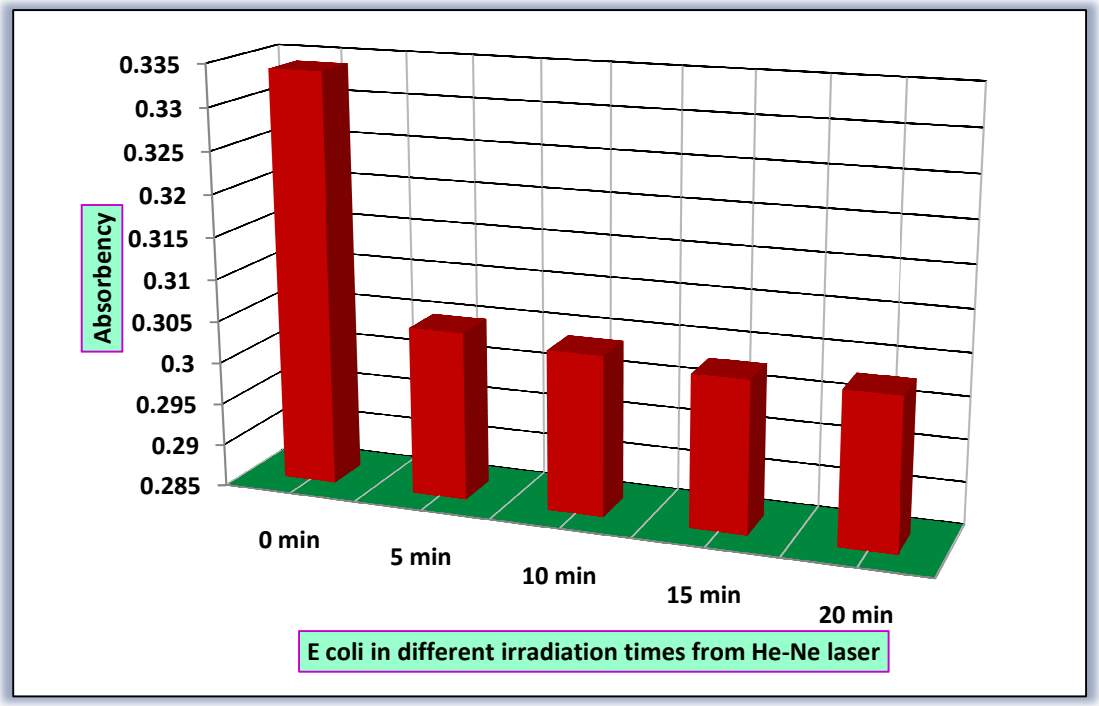


Figure (4-17): Represents Effect of He-Ne laser in different irradiation times on *E. coli.*

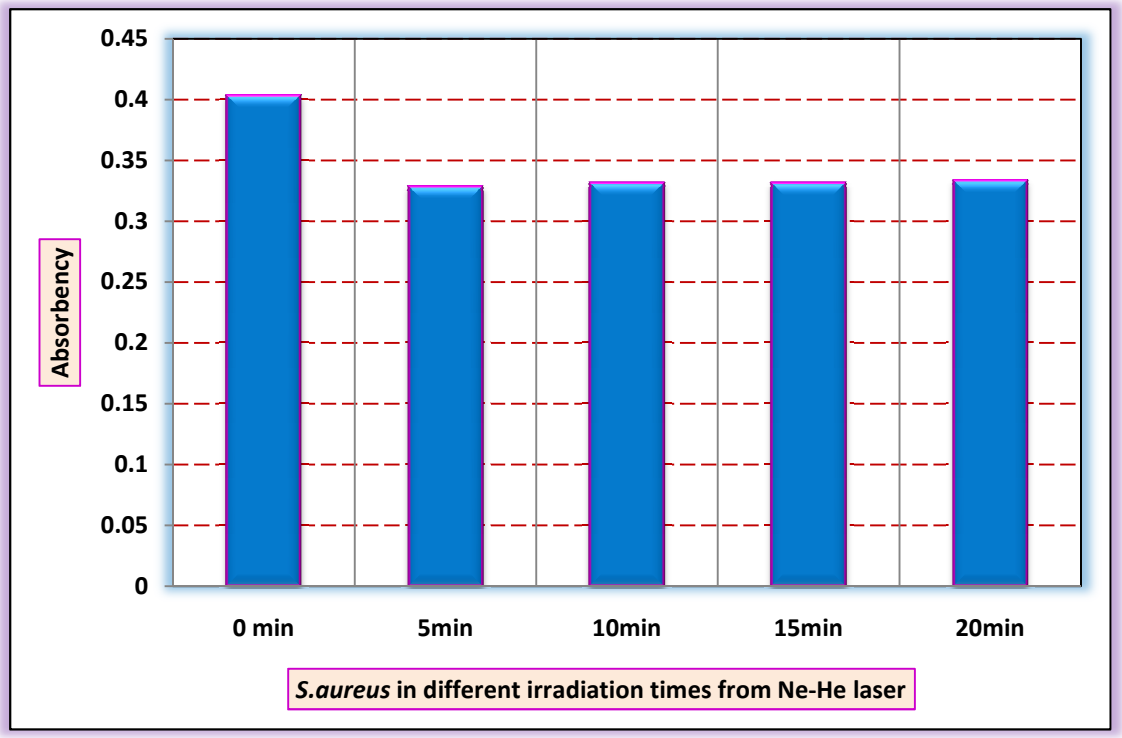


Figure (4-18): Represents Effect of He-Ne laser with in different irradiation times on *S. aureus.*

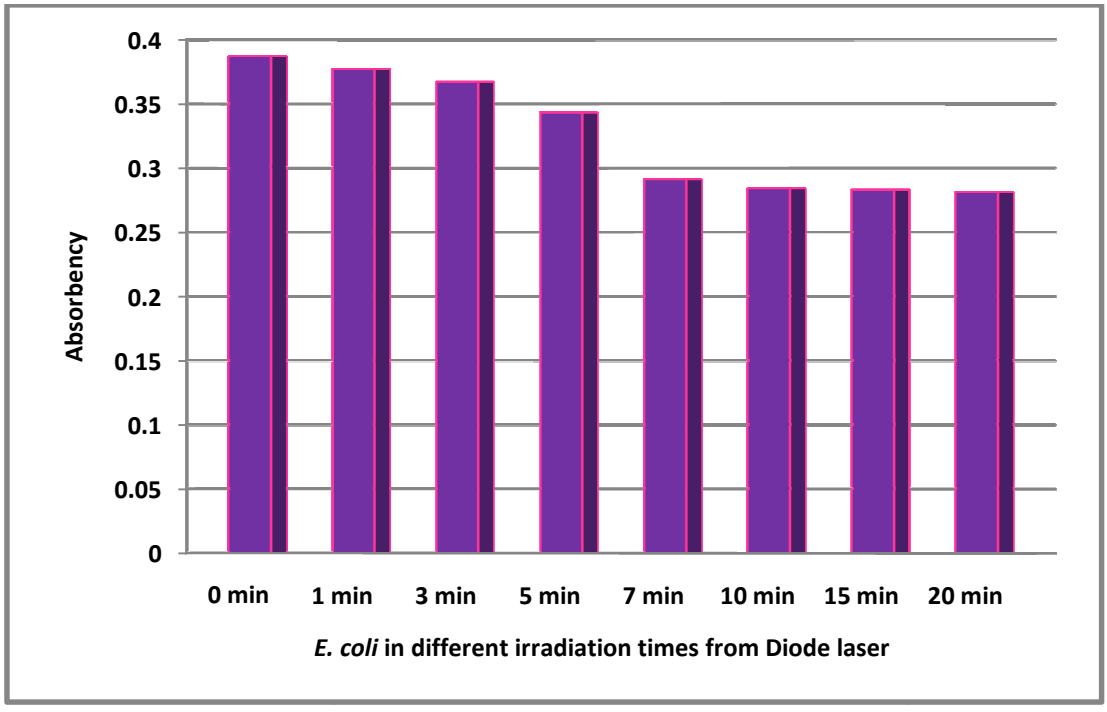


Figure (4-19): Represents Effect of Diode laser in different irradiation times on *E. coli.*

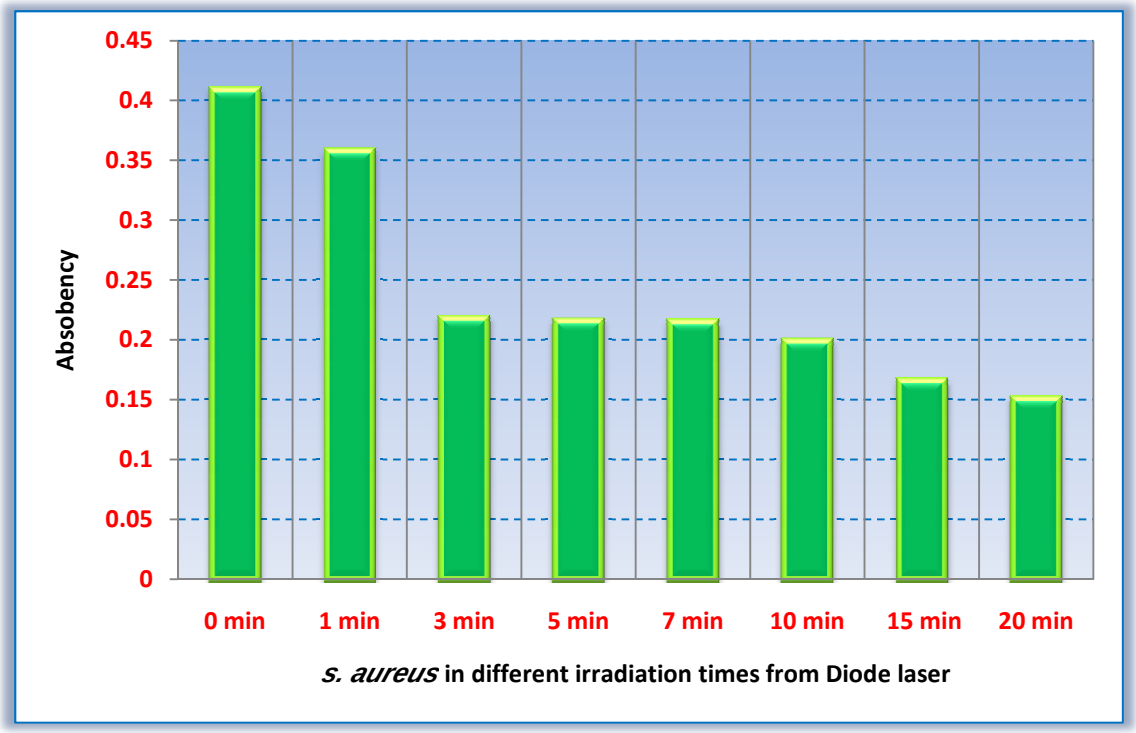


Figure (4-20): Represents Effect of Diode laser in different irradiation times on *S. aureus.*

Current study detected inhibitory action of diode laser on *S. aureus* and this agree with study of Ismail *et al* (2012) who show that diode laser light reduce *S. aureus* growth but not effect on the enzymes production (Catalase and Coagulase), and the fermentation of mannitol of these isolate [13].

Present research determine bacterial inhibition increase with increase time of exposure to lasers radiation and this in consent with other studies [76,77, 16].

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 He-Ne 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 [12].

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 singlet oxygen [71].

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 [12].

4.6. Mutual Effect of Laser Irradiation and Nanoparticles on Bacterial Species

The dual effect of laser irradiation (He-Ne with 5 mW, and Diode with 50 mW) with Nanoparticles (AgNPs and ZnO NPs) on *E. coli* and *S. aureus* can be shown in the following sections.

4.6.1. Companied Effect of He-Ne laser (5 mW) and Nanoparticles on tested Bacterial Species

This section can be divided into two parts which are:

A- Effect He-Ne laser (5 mW) with AgNPs on Bacterial Species

The effect of He-Ne laser (5 mW) with AgNPs on *E. coli* and *S. aureus* can be seen in the figure (4-21) and figure (4-22) consequently. Figures (4-21,4-22) shows, increase inhibition of bacterial species (mainly *S. aureus*) by lower concentration of nanoparticles with He-Ne laser (10 min).

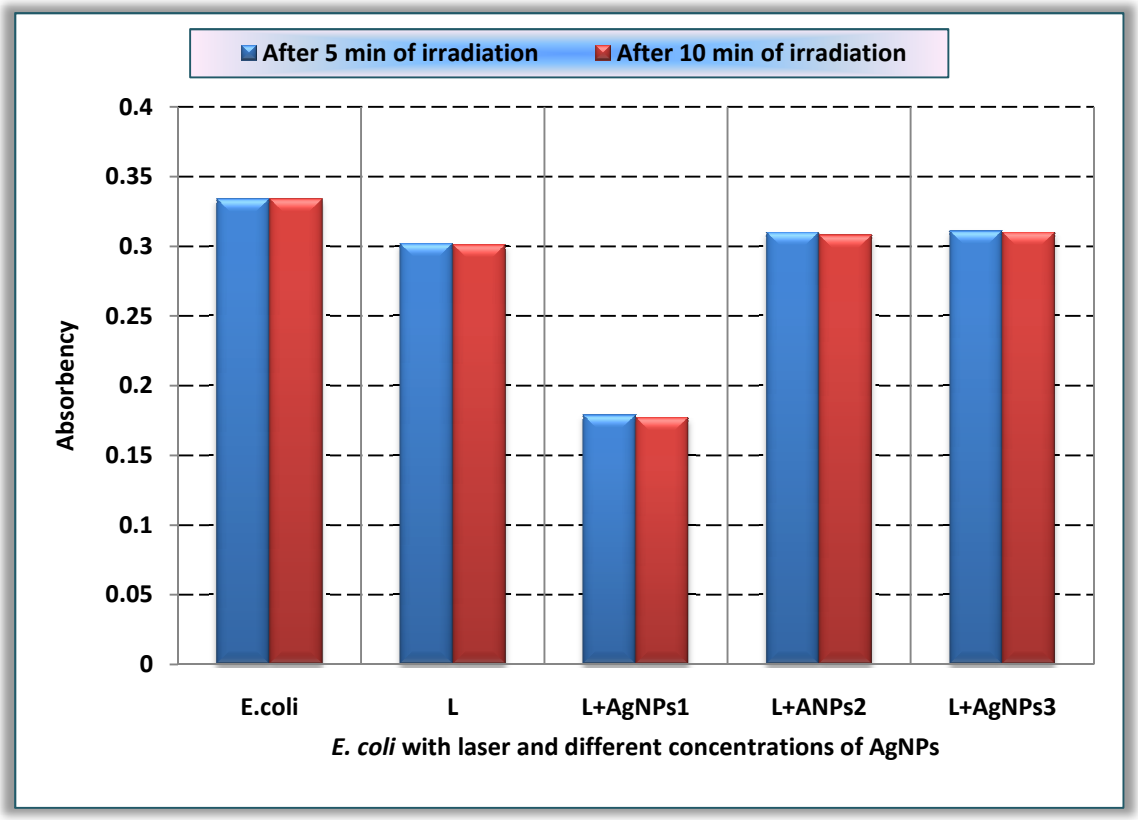


Figure (4-21): Represents Effect of He-Ne in two irradiation times with AgNPs in different concentrations on *E. coli*.

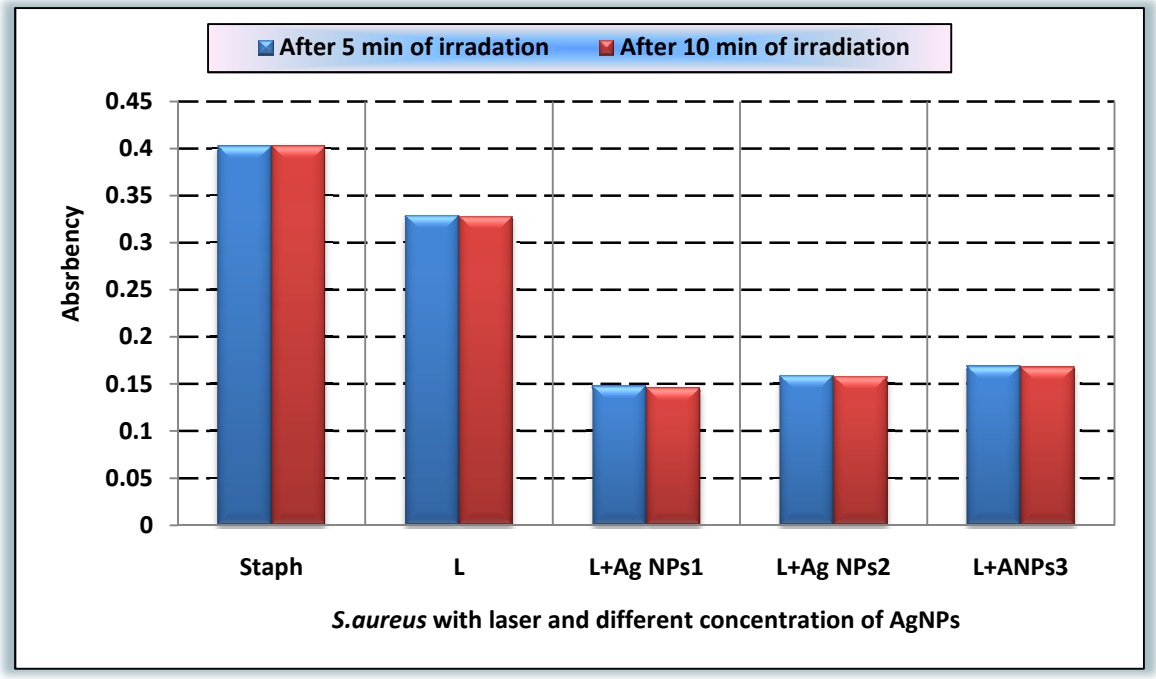


Figure (4-22): Represents Effect of He-Ne in two irradiation times with AgNPs in different concentrations on *S. aureus*.

B- Effect He-Ne laser (5 mW) with ZnO NPs on Bacterial Species

The effect of He-Ne laser (5 mW) with ZnO NPs on *E. coli* and *S. aureus* can be seen in the figure (4-23) and figure (4-24) respectively. Result shows, increase inhibition of bacterial species (primarily *S. aureus*) by lower concentration of nanoparticles with He-Ne laser (10 min).

4.6.2. Companied Effect of Diode Laser (50 mW) and Nanoparticles on tested Bacterial Species

This section was divided into two parts which are:

A- Effect Diode Laser with AgNPs on Bacterial Species

The effect of Diode laser (50 mW) with AgNPs on *E. coli* and *S. aureus* can be seen in the figure (4-25) and figure (4-26) consequently. Result shows, increase inhibition of bacterial species (primarily *S. aureus*) by lower concentration of nanoparticles with Diode laser (10 min).

B- Effect Diode Laser with ZnO NPs on Bacterial Species

The effect of diode laser (50 mW) with ZnO NPs on *E. coli* and *S. aureus* can be seen in the figure (4-27) and figure (4-28) respectively. Result shows, increase inhibition of bacterial species (primarily *S. aureus*) by lower concentration of nanoparticles with Diode laser (10 min).

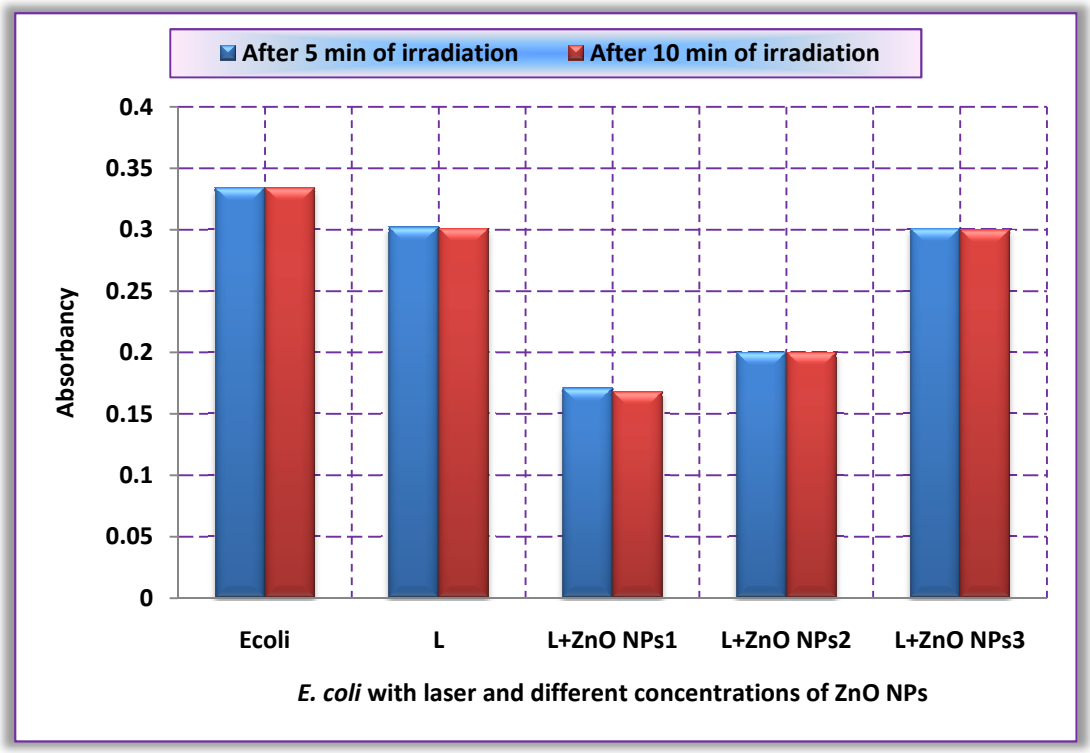


Figure (4-23): Represents effect of He-Ne in two irradiation times with ZnO NPs in different concentrations on *E. coli*.

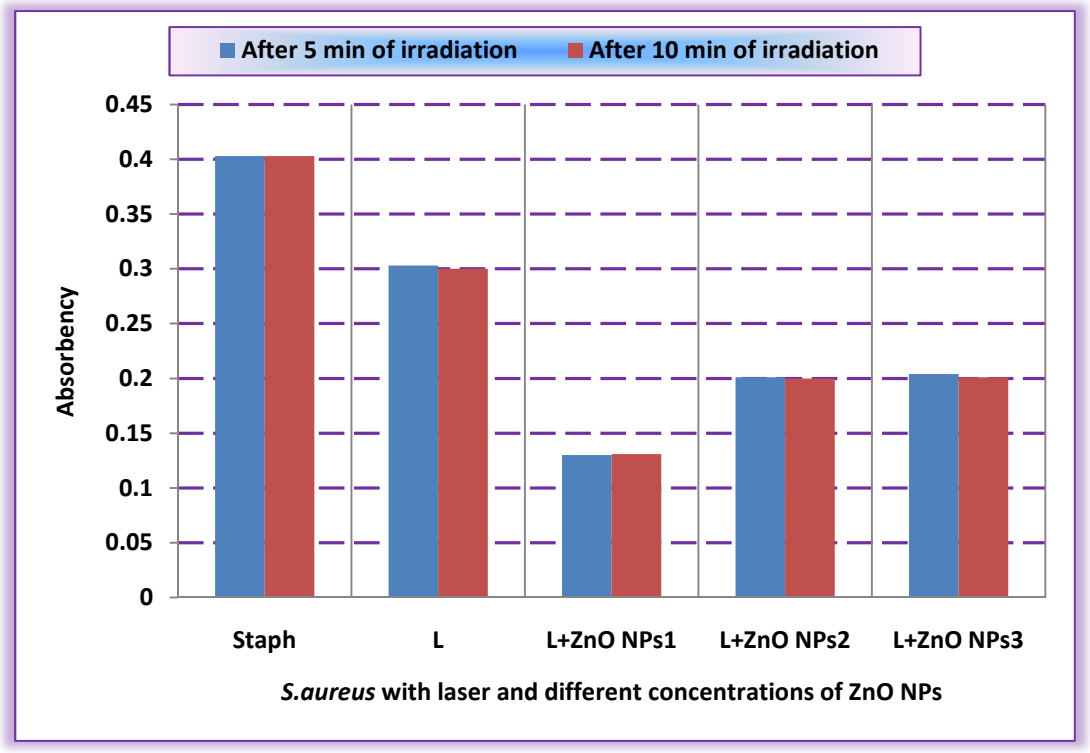


Figure (4-24): Represents effect of He-Ne in two irradiation times with ZnO NPs in different concentrations on *S. aureus*.

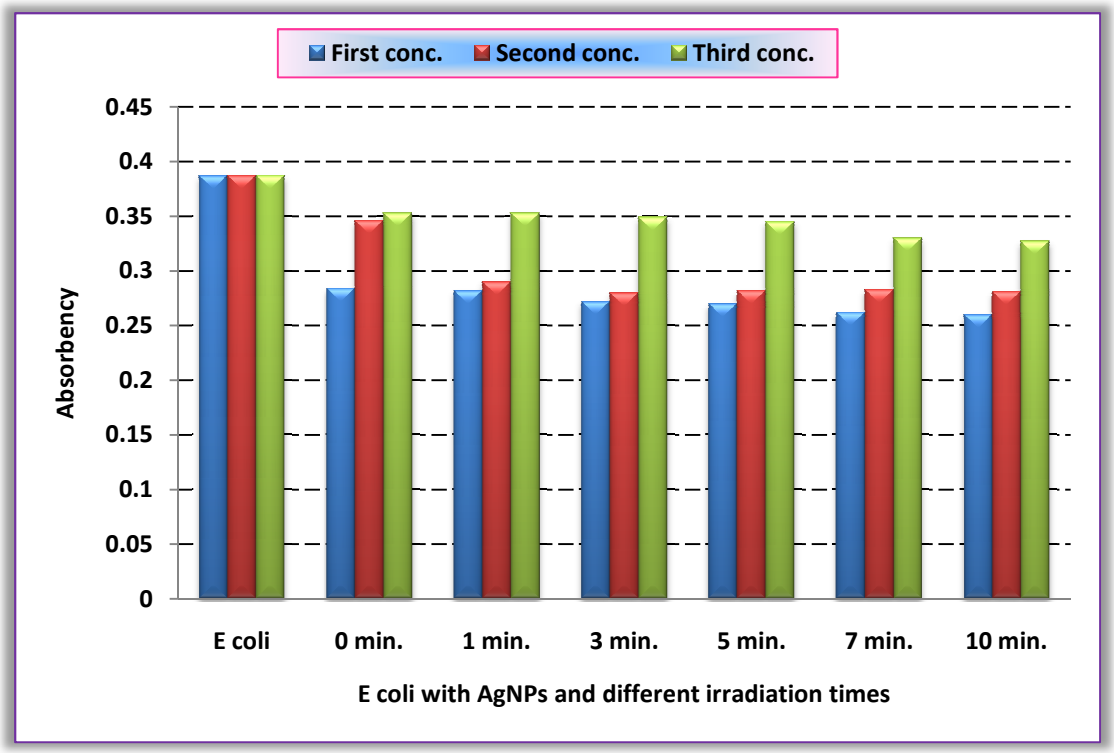


Figure (4-25): Represents Effect of Diode laser in different irradiation times with AgNPs in different concentrations on *E.coli*.

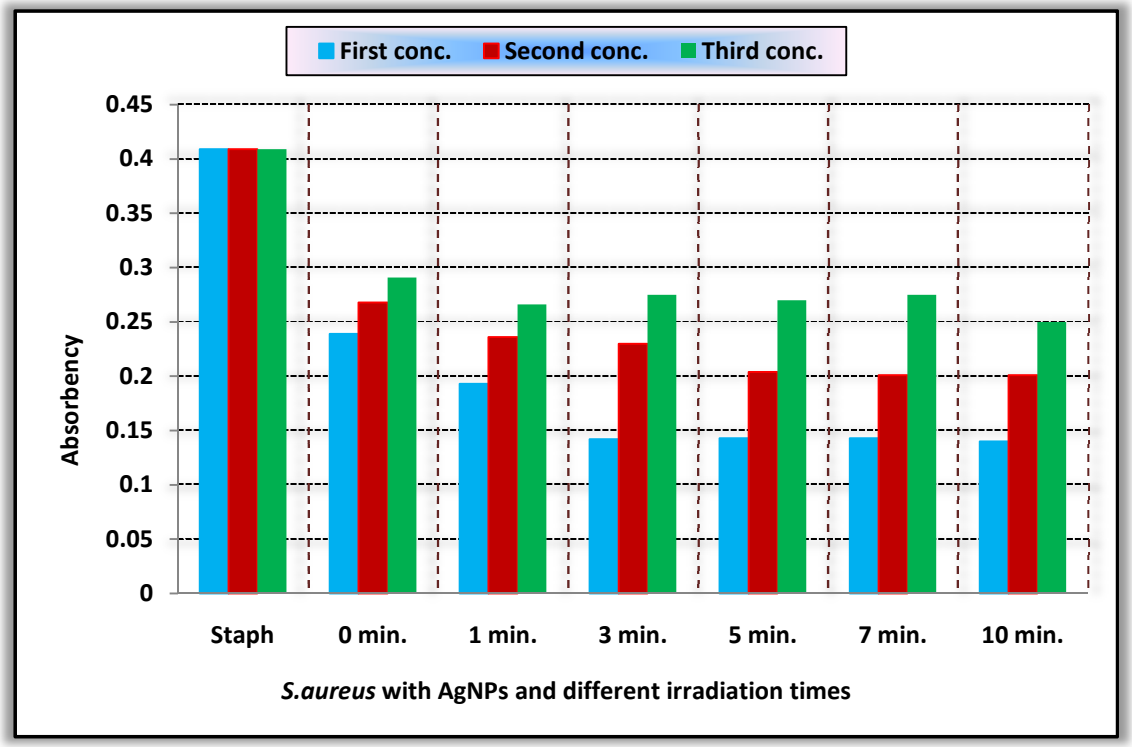


Figure (4-26): Represents Effect of Diode laser in different irradiation times with AgNPs in different concentrations on *S. aureus*.

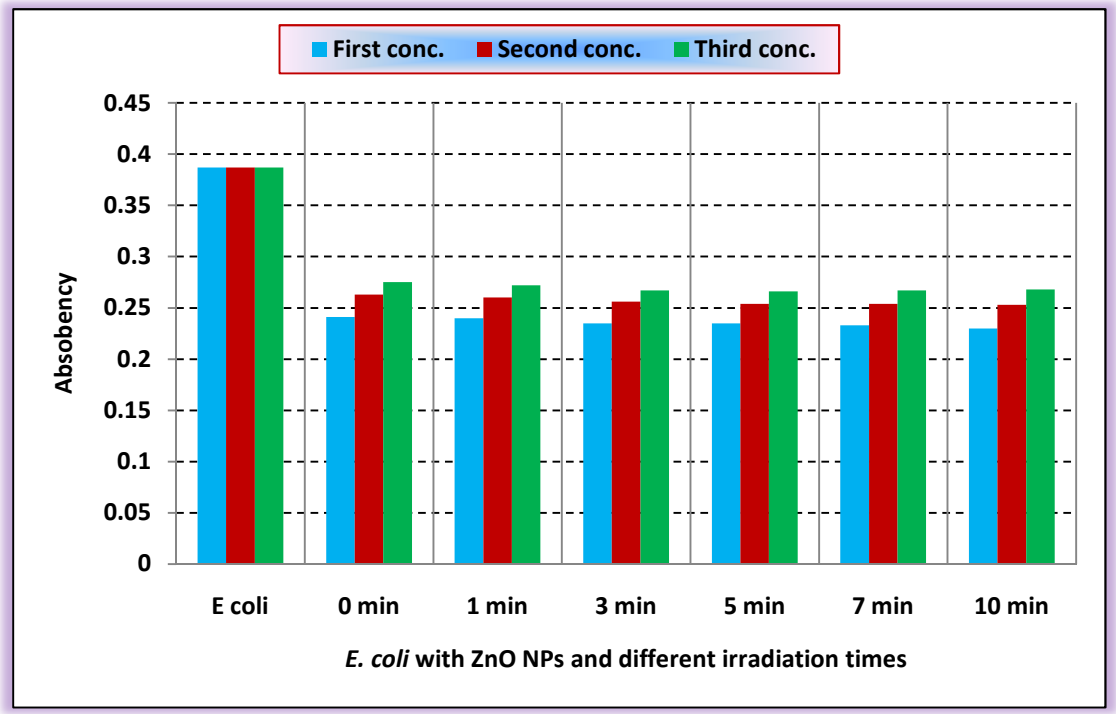


Figure.(4-27): Represents Effect of Diode laser in different irradiation times with ZnO NPs in different concentrations on *E. coli*.

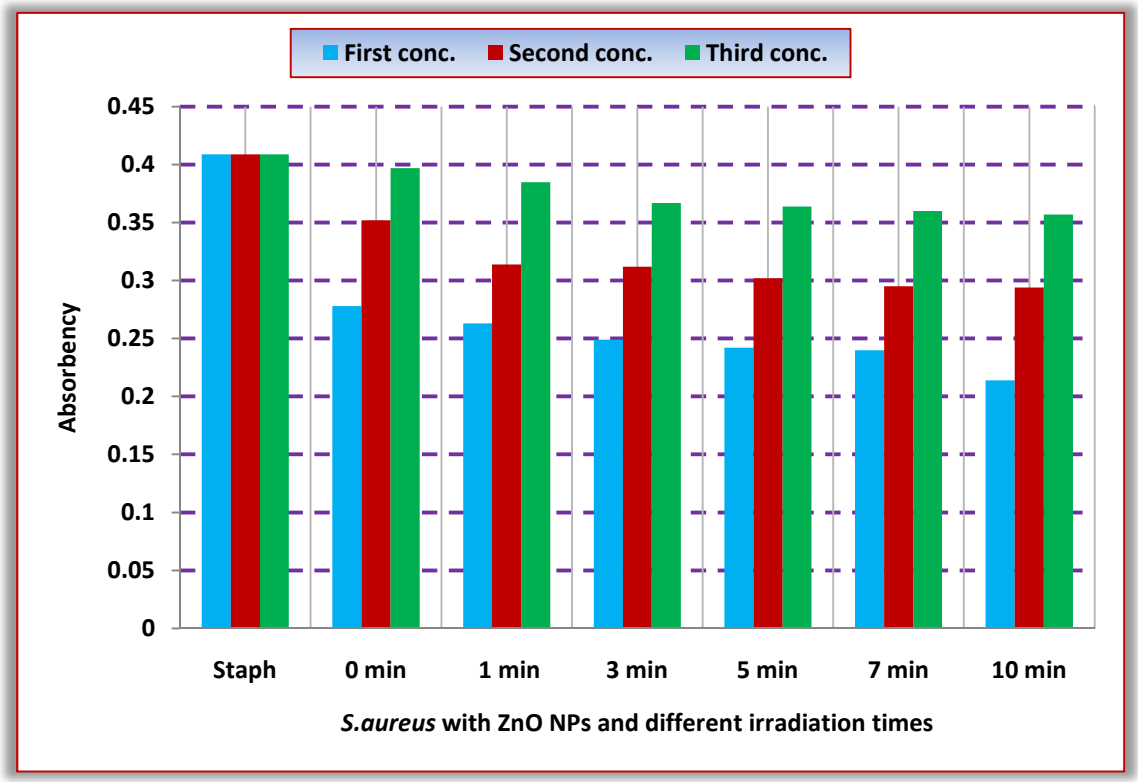


Figure (4-28): Represents effect of Diode laser in different irradiation times with ZnO NPs in different concentrations on *S. aureus*.

The dual antibiotic action of lasers and nanoparticles depend on bacterial species, lasers properties, time of exposure to lasers, type and concentration of nanoparticles [78,13].

In present research, nanoparticles with He-Ne laser more effect on bacterial species (mainly *S. aureus*) than those with diode laser. In the study current, the dual action of Diode 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 24hr of incubation [18].

Zharov *et al*.,(2006) show that syneregestic action of lasers and nanoparticles relate to strongly absorb laser irradiation by nanoparticles and this absorbed energy transforms quickly into heat, which causes damage to the bacterium through local overheating effects. If many nanoparticles attach to the bacterial surface, there will be multiple damage sites with possible overlapping thermal spots from the particles within nanoclusters [79].

Results showed that high concentration of nanoparticles have the highest inhibitory effect on tested bacterial species (especially on *S. aureus*) whereas inhibitory action of Diode laser on tested bacterial species are more than He-Ne laser with increasing the time of irradiation until reach 20 min. This effect was due to the high power of Diode laser (with 50 mW) than He-Ne laser (with 5 mW) regardless of wavelength because they were neared (632.8 nm for He-Ne and 650 nm for Diode laser). Also result determined increase inhibition of bacterial species (primarily *S. aureus*) by high concentration of nanoparticles with lasers at 10 min irradiations time.

4.7. Conclusions:

1- AgNPs and ZnO NPs are inhibit or kill *S. aureus* and *E. coli*.

2- The inhibition or killing of bacteria were increased when the concentrations of nanoparticles were amplified.

3- The effect of AgNPs on *S. aureus* and *E. coli* was more than the effect of ZnO NPs.

4- The results investigation that He-Ne laser and diode laser have inhibitory action on *E. coli* and *S. aureus*.

5- Sensitivity of *E. coli* to laser enhance with increase time of exposure to irradiation.

6- The dual antibiotic action of He-Ne laser and nanoparticles significantly lead to inhibition of the bacterial growth.

7- *S. aureus* more effected by laser or nanoparticles than *E. coli*.

4.8. Recommendations:

Surgical tools and rooms can be sterilized by using:

1- The nanoparticles used (with high concentrations and small particle size $\leq$ 20 nm).

2- High power laser (power $\geq$ 50 mW) and increase irradiation times more than 20 minutes.

3- The dual effect of high concentrations nanoparticles, high power laser and long irradiation times can further increase the killing of bacteria.

4.9. Future work:

1- Study the effect of nano gold on bacteria.

2- Using another lasers with high power on bacteria.

3- Using more irradiation times from laser.

4- Using another species of bacteria.

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الخلاصة

هذه الدراسة هي محاولة لتوضيح كيفية استخدام الليزر أو الجسيمات النانوية لأوكسيد الزنك (ZnO NPs) والجسيمات النانوية للفضة (AgNPs) كل منها على حدة، كمضاد بكتيري ضد المكورات العنقودية الذهبية (*S. aureus*) التي هي بكتيريا إيجابية لصبغه غرام والإشريكية القولونية (*E. coli*) التي هي بكتيريا سالبة لصبغه غرام وكذلك دراسة النتيجة المؤثرة من امتصاص طاقة الليزر بواسطة هذه الجسيمات النانوية لقتل أو تثبيط نمو البكتيريا.

تم تحضير AgNPs بواسطة طريقة بيولوجية وتذرية الليزر للفضة في الماء المقطر في حين أن الجسيمات النانوية لأوكسيد الزنك حضرث بواسطة الطريقة الكيميائية.

تم أستخدام المجهر الإلكتروني إلنفاذ (TEM) والمجهر الإلكتروني الفاحص (SEM) و تشتت الطاقة الأشعة السينية الطيفي (EDS) ومنطقة مطياف الأشعة فوق البنفسجية البصرية لفحص خصائص الجسيمات النانوية. كذلك تم استخدام ليزر He-Ne وDiode (مع قدرات تشعيع مختلفة وأطوال موجية مختلفة و أزمان مختلفة) لقتل أو تثبيط نمو *S. aureus* و *E. coli*.

وأظهرت النتائج أن التركيز العالي للجسيمات النانوية له أعلى تأثير مثبط على الأنواع البكتيرية المختبرة (وخاصة على *S. aureus*) في حين أن فعالية التثبيط لليزر الدايود لانواع البكترية المستخدمة هو اعلى مقارنة مع ليزر الهيليوم-نيون مع زيادة زمن التشعيع و لحد الوصول الى 20 دقيقة. هذا التأثير ناتج من القدره العالية لليزر الدايود (50 mW) مقارنتا مع ليزر الهيليوم-نيون (5 mW).

أيضا النتائج أظهرت زيادة تثبيط الأنواع البكتيرية (في المقام الأول*S. aureus*) عن طريق تركيز أعلى للجسيمات النانوية مع أشعة الليزر في 10 دقائق من زمن التشعيع.

نستنتج ان زيادة تثبيط أو قتل البكتيريا تم بزيادة تركيز الجسيمات النانوية. بالإضافة إلى ذلك، حساسية البكتيريا لليزر زادت مع زيادة وقت التعرض وزيادة قدرة الليزر.

وزارة التعليم العالي
والبحث العلمي
جامعة القادسية
كلية التربية
قسم الفيزياء

دراسة في تأثير الليزر والجسيمات النانوية على أنواع مختلفة من البكتريا

رسالة مقدمة إلى

مجلس كلية التربية – جامعة القادسية

كجزء من متطلبات نيل درجة الماجستير

في علوم في الفيزياء

من قبل

صفاء عبد الامير ياسين

بكالوريوس علوم في الفيزياء – 2000

بإشراف

أ. د. رعد شاكر النائلي

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