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Effect of Gamma, Beta, Alpha irradiation and effect of Semiconductor laser on *Serratia marcescens* (*in vitro*)

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Abstract : This work evaluated the effect of gamma , beta ,alpha and Semiconductor laser irradiation on *Serratia marcescens* The experiment included a control and 5 doses of each gamma , beta and alpha irradiation ,which ranged(1.317×10^{-4} -0.320µSv) and (10.573-96.950µSv) respectively.The total effect of Gamma , Beta and Alpha irradiation on *S. marcescens* viability was counted after exposure to (^{137}Cs , ^{90}Sr ,^{60}Co , ^{241}Am , ^{22}Na) isotope for 3 hr. respectively, the percentage of killing was highly 83%, 85%, 75%, 90%, 82% respectively . The viable cells was fewer than control (without exposure to irradiation) . This work evaluated also the effect of Semiconductor laser on *S. marcescens* . experiment included a control and triplicate exposed to Semiconductor laser in power 5 mW , in Wavelength 650 nm . The effect of Semiconductor laser on the viability of *S. marcescens* was counted and percentage of killing was counted, the number of viable cells of *S. marcescenc* was fewer than control (without exposure to laser), but percentage of killing was high. Gamma, Beta, alpha irradiation and semiconductor laser was efficient to killing *S. marcescens* that cause many infection to human.

Keywords: *S. marcescens*, laser, irradiation, type of decay.

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Introduction

Serratia marcescens is a member of the genus *Serratia*, which is a part of the family *Enterobacteriaceae*. Currently 14 species of *Serratia* are recognized within the genus, eight of which are associated with human infection. of the eight species implicated in clinical infection *S. marcescens, S. liquefaciens* and *S. odorifera* are best known, all *Serratia* species, *S. marcescens* is the most common clinical isolate and the most important human pathogen (1). As members of the *Enterobacteriaceae*

Family, *Serratia spp* are motile, non-endospore forming Gram-negative rods. *Serratia* are isolated from bloodstream and wound sites or from respiratory and urinary sites (2).

S.marcescens is generally an opportunistic pathogen causing infect-ions in immunocompromised patients. Among the possible pathogenicity factors found in *Serratia* strains are the formation of fimbriae, the production of potent siderophores, the presence of cell wall antigens, the ability to resist to the bactericidal action of serum, and the production of proteases, the ability of *S. marcescens* cells to attach to human buccal epithelial cells or to the human urinary bladder surface **(**3).

Phenotypically *Serratia* is one of the easiest genera to differentiate with in the *Enterobacteriaceae* family.

Serratia usually produce extracellular deoxyribonuclease

(DNase), gelatinase and lipase and are resistant to the antibiotics colistin and cephalothin (4).

The healthy human being does not often become infected by *Serratia*, whereas the hospitalized patient is frequently colonized or infected.*S. marcescens* is the only known nosocomial species of *Serratia* are occasionally isolated from clinical specimens. *Serratia* infections do not differ from infections by other opportunistic pathogens, respiratory tract infection and colonization of intubated patients, urinary tract infection and colonization of patients with indwelling catheters, surgical wound infection or superinfection and septicemia in patients with intravenous catheterization or complicating a local infection (osteomyelitis, ocular or skin infections) Meningitis, brain abscesses, and intraabdominal infections are more exceptional **(**3). Gamma irradiation is a physical means of decontamination, because it kills bacteria by breaking down bacterial DNA, inhibiting bacterial division. Energy of gamma rays passes through hive equipment, disrupting the pathogens that cause contamination (5). Radiation sterilization, as a physical cold process, has been widely used in many developed and developing countries for the sterilization of health care products. A historical review shows clearly that ionizing radiation was used extensively for the treatment of many types of infections before the advent of antibiotics (6). It is well known that exposure of microbial cells to ionizing radiation presents an additional stress to the cells which tends to disturb their organization. Nucleic acids, especially DNA, are the primary target for cell damage from ionizing radiation. Breaks in the DNA chain disrupt function of the molecule in several ways (7). Because of these shortages of antimicrobials, the use of laser irradiation has become a topic of much interest and is a promising field in periodontal therapy (8). Antimicrobial effect of laser is considered as a safe coadjutant in nonsurgical treatment of gingivitis, as it has been proved to reduce the signs of inflammation and microbial infection without any harmful effects on adjacent periodontal tissues (9, 10). Semiconductor laser is capable of decontaminating implant surfaces. Surface characteristics determine the necessary power density to achieve a sufficient bactericidal effect. The rapid heat generation during laser irradiation requires special consideration of thermal damage to adjacent tissues (11). Due to the advantages of semiconductor laser such as small body, light weight, long life span, high efficiency, it has been used widely in the medical fields (12) .The aim of this primary study was to detect the effect of gamma, Beta Alpha irradiation and effect of Semiconductor laser on the viability of *S. marcescens*.

Materials and methods

Bacterial isolates

A total of 10 isolates of *S. marcescens* were collected from different samples from patients who were admitted to Baghdad hospitals in 2015.These isolates were identified by conventional biochemical reactions.

Effect of Gamma, Beta, Alpha Irradiation on *S. marcescens* isolates.

S. marcescens cultivation was done according to Tramps *et al.*, (13) with some modifications as follows: The

irradiation facility used was gamma (γ) irradiation, Beta, Alpha irradiation and of Semiconductor laser in different dose and different energy for 3hr. . The *S. marcescens* isolates was grown in Nutrient broth for 24 h. on shaker (150 rpm) at 30ºC. The well grown bacterial culture was centrifuged at 8000 rpm for 15minutes. The supernatant was decanted and the pellets were suspended in sterile saline. The suspended cells were collected in a clean sterile flask to form pool. The bacterial suspension of the pool (5ml) was distributed in clean sterile screw cap test tubes and exposed to different doses of Gamma, Beta, Alpha radiation using triplicates for each dose. The non-irradiated control and the irradiated cultures were plated on the surface of Trypton soy agar plates. Also take 1 ml of this solution was exposed to Semiconductor laser (continuous laser) ,output for S.C. laser (ʰυ =532 ±10) nm . the output power = < 200 mW . for S.C. laser ʰυ = (650±10) nm , the output power =< 100 mW . energy density or power density for(ʰυ =532 ±10) nm , power density is 200 mW /cm^2 .but in S.C. laser ʰυ = (650±10) nm ,power density is 100 mW/ cm^2 . .spot size of exposure to laser light =1 cm^2. in different time (5 , 10 , 20 , 30) min , in comparison to control group (without exposure to laser), Each run was done in triplicate, inoculated in Trypton soy agar . and the viable count, percentage of killing was determined .

The percentage of killing calculated from equation below:

$$\text{Percentage of Killing} = \frac{\text{Control} - \text{treated}}{\text{Control}} * 100$$

Statistical Analysis

The Statistical Analysis System-SAS (14). Program was used to compare the effect of difference factors in study parameters. (14) Chi-square test was used to significant compare between groups in this study. The difference between two groups. $P< 0.05$ was considered as significant difference and $P< 0.01$ as highly significant difference.

Results and Disscusion

The lethal effect of ionizing radiation on microorganisms, as measured by the loss cells of colony-forming ability in Nutrient medium, has been the subject of detailed study. Much progress has been made towards identification of the mechanism of inactivation, but there still remains considerable doubt as to the nature of the critical infection involved, although it seems certain that lethality is primarily the consequence of genetic damage (15). Soon after the discovery of lasers in the 1960s it was realized that laser therapy had the potential to improve wound healing and reduce pain, inflammation and swelling. In recent years the field sometimes known as photobiomodulation has broadened to include light-emitting diodes and other light sources, and the range of wavelengths used now includes many in the red and near infrared. The term "low level laser therapy" or LLLT has become widely recognized and implies the existence of the biphasic dose response or the Arndt-Schulz curve. This study will cover the mechanisms of action of LLLT at a cellular and at a tissular level and will summarize the various light sources and principles of dosimetry that are

employed in clinical practice. The range of diseases, injuries, and conditions that can be benefited by LLLT will be summarized with an emphasis on those that have reported randomized controlled clinical trials. Serious life-threatening diseases such as stroke, heart attack, spinal cord injury, and traumatic brain injury may soon be amenable to LLLT therapy(16).This study aims to prove the effect of Gamma, Beta, Alpha irradiation and effect of Semiconductor laser directly on the *S. marcescens* ,exposed to different doses ,different energy of irradiation, these cells determined using count of colony and percentage of killing after exposed to Cs^{137} , Co^{60} , Sr^{90}, Na^{22} . in dose Cs^{137} $0.3863*10^{-8}$, Co^{60} $1.826*10^{-8}$, $Sr^{90}1.973*10^{-8}$, Na ($0.31993*10^{-8}$ Gamma and $1.4157*10^{-8}$ Beta) , $0.31993*10^{-8}$ respectively .The viability of these cells and percentage of killing showed in Table 1 .and exposed to different time (5,10,20,30) min of Semiconductor laser in power 5mW , wavelenghth 650 nm , the viability of these cells determined using count of *S. marcescens* colony Table 2. The results showed when increase exposure *S. marcescens* to Semiconductor laser and Gamma ,Beta ,Alpha irradiation, and Semiconductor laser , percentage of killing was higher than control because effect directly or indirectly on cell membrane , DNA , cytoplasmic membrane ,cause damage to bacterial cells ,and cause death. By rays generated from Isotopes and laser.

Table (1): The percentage of killing of *S. marcescens* colony after exposure to Gamma, Beta, Alpha irradiation, and Dose, Energy and Activity of Isotope.

Source isotope	Dose (MSV)	Energy(keV)	Type of irradiation	Activity µci	Killing ration %
Cs	1.77634 Gamma 274.54 Beta	661.66+ 515.97	Beta+ Gamma	1	83
Sr	63.09995286	198.8	Beta	1	85
Co	10.54262083 Beta $1.3172*10^{-4}$ Gamma	117324+ 31813	Beta + Gamma	1	75
Na	$1.4157*10^{-4}$	1274.53	Gamma	1	82
Am	$0.31993998*10^{-4}$ Gamma $1.4157*10^{-4}$ Alpha	59.54	Gamma	1	90

Gamma rays cause damage at a cellular level and are penetrating, causing diffuse damage throughout the body. However, they are less ionising than alpha or beta particles, which are less penetrating. Low levels of gamma rays cause a stochastic health risk, which for radiation dose assessment is defined as the probability of cancer induction and genetic damage. High doses produce deterministic effects, which is the severity of acute tissue damage that is certain to happen. Gamma radiation is often used to kill living organisms, in a process called irradiation. Applications of this include the sterilization of medical equipment (as an alternative to autoclaves or chemical means), the removal of decay-causing bacteria from many foods and the prevention of the sprouting of fruit and vegetables to maintain freshness and flavor (17). Radiation-induced ionizations may act

directly on the cellular component molecules or indirectly on water molecules, causing water-derived radicals. Radicals react with nearby molecules in a very short time, resulting in breakage of chemical bonds or oxidation (addition of oxygen atoms) of the affected molecules. The major effect in cells is DNA breaks. Since DNA consists of a pair of complementary double strands, breaks of either a single strand or both strands can occur. However, the latter is believed to be much more important biologically. Most single-strand breaks can be repaired normally thanks to the double-stranded nature of the DNA molecule (the two strands complement each other, so that an intact strand can serve as a template for repair of its damaged, opposite strand). In the case of double-strand breaks, however, repair is more difficult and erroneous rejoining of broken ends may occur. These so-called misrepairs result in induction of mutations, chromosome aberrations, or cell death. Deletion of DNA segments is the predominant form of radiation damage in cells that survive irradiation. It may be caused by misrepair of two separate double-strand breaks in a DNA molecule with joining of the two outer ends and loss of the fragment between the breaks or the process of cleaning (enzyme digestion of nucleotides -the component molecules of DNA) of the broken ends before rejoining to repair one double-strand break (18). Despite their cancer-causing properties, gamma rays are also used to treat some types of cancer, since the rays kill cancer cells also. In the procedure called gamma-knife surgery, multiple concentrated beams of gamma rays are directed to the growth in order to kill the cancerous cells. The beams are aimed from different angles to concentrate the radiation on the growth while minimizing damage to surrounding tissues (17). A previous study by Wen *et al*. (19) used Lycium fruit, popular traditional Chinese medicine and food supplement generally is ingested uncooked, was exposed to several doses of gamma irradiation (0–14 kGy) to evaluate decontamination efficiency, changes in chemical composition, and changes in sensory characteristic. lycium fruit specimens contained microbial counts of 3.1×10^{3}–1.7×10^{5} CFU/g and 14 kGy was sufficient for microbial decontamination Before irradiation, the main microbe isolated from lycium fruit was identified as a strain of yeast, *Cryptococcus laurentii*, Gram-positive spore-forming bacterium, *Bacillus cereus C. laurentii* and *B. cereus* was approximate -ly 0.6 and 6.5 kGy, respectively, the D_{10} doses of *C. laurentii* and *B. cereus* was approximately 0.6 and 1.7 kGy, respectively. Also study by Nguyen *et al*. (20) proved used Gamma irradiation from Cobalt 60 sources has been to terminally sterilize bone allografts for many years. Gamma radiation adversely affects the mechanical and biological properties of bone allografts by degrading the collagen in bone matrix. Specifically, gamma rays split polypeptide chains. In wet specimens irradiation causes release of free radicals via radiolysis of water molecules that induces cross-linking reactions in collagen molecules. These effects are dose dependent and give rise to a dose-dependent decrease in mechanical properties of allograft bone

when gamma dose is increased above 25 kGy for cortical bone or 60 kGy for cancellous bone. This has resulted in the application of doses ranging from 15 to 35 kGy. Study by Lamb *et al*. (21) used low dose Gamma irradiation was an effective method for reducing and killing *Staphylococcus aureus* .The results proved By Patel (22). As the UV light exposure time increased, the bacterial growth decreased. However, it was not expected that *S. marcescens* bacteria exposed to ultraviolet light for 2 minutes would result in almost complete mortality. Concluded that the titanium dioxide sunblocks were more effective in protecting *S. marcescens* against ultraviolet light than oxybenzone. the effects of short term ultraviolet light exposure on *S. marcescens*, and determined that titanium dioxide is more effective than oxybenzone in protecting *S.marcescens* against UV radiation.There has been an increased interest in control of microbial growth, especially in light of single and multiply drug-resistant "superbugs". Most students are also aware, at some level, of the side effects resulting from exposure to ultraviolet (UV) radiation. This compares the level of UV sensitivity displayed by *S. marcescens* (a commonly-occurring, mildly pathogenic, pigmented organism) to that displayed by *Deinococcus radiodurans* (unrelated, non-pathogenic, pigmented organism). Inoculate TSA plates with organism, irradiate with UV light for specified times, and subsequently score plates for numbers and phenotypes of organism. Results demonstrate a dose-dependent sensitivity of *S. marcescens* cultures to UV-irradiation, with lethality as the most commonly scored phenotype. By contrast, similarly treated *Deinococcus radiodurans* cultures are resistant to the effects of UV irradiation over the length of exposure tested. The results are discussed relative to possible differences in cell-specific management of oxidative stress (23). Experimental systems to investigate the influence of various environmental parameters on the efficacy of UV germicidal irradiation (UVGI) for deactivating airborne microorganisms. The effect of the composition of the suspending medium on the size and UVGI susceptibility of *Serratia marcescens* IS effected. *S. marcescens* suspended in water-only medium was the most susceptible to UVGI, followed by those in serum-only medium, the choice of suspending medium influenced both size and UVGI susceptibility of *S. marcescens*. These data are valuable for making comparisons and deciding on the use of an appropriate medium for various applications (24). Many hypotheses have been proposed and tested regarding the mechanism of cell damage by radiation. Some scientists proposed the mechanism thought 'radiotoxins' that are the toxic substances produced in the irradiated cells responsible for lethal effect. Others proposed that radiation was directly damaging the cellular membranes. In addition, radiation effects on enzymes or on energy metabolism were postulated. The effect on the cytoplasmic membrane appears to play an additional role in some circumstances (15).

Table (2): The percentage of killing *S. marcescence* colony (1). after exposure to Semiconductor laser .

	percentage of cell killing exposed to semiconductor laser			
	5min		10 min	
	Viable cells	Precentage of killing %	Viable cells	Precentage of killing %
S1	23	92.3	20	93.3
S2	22	92.6	20	93.3
p-value	NS*		NS	
	20min		30min	
	Viable cells	Precentage of killing %	Viable cells	Precentage of killing %
S1	10	96.6	8	98
S2	9	97	8	98
p-value	NS		NS	

***NS=no significant**

In Table 2, number of viable cells and percentage of killing after exposure to Semiconductor laser in (5, 10, 20, 30) minute, in powe 5Mw , in Wavelenghth 650 nm , the results showed when increase exposure *S. marcescence* to semiconductor laser the percentage killing of this bacteria was increased, because effect directly or indirectly on cell membrane , DNA , cytoplasmic membrane , by heat generated from semiconductor laser . A previous study done by Pirnat *et al*.(25) indicate that the primary interference of cell death appears to be the interaction between near-infrared spectrum laser light and the bacterial micro-environment, most likely in the form of heating, thus the present study suggest the same action on *S.marcescence.* Also, a previous study by Luan *et al.* (26). Indicate that antimicrobial effect of laser is considered as a safe coadjutant in nonsurgical treatment of gingivitis, as it has been proved to reduce the signs of inflammation and microbial infection without any harmful effects on adjacent periodontal tissues. A light from low – power laser with an appropriate wavelength , it will be excited to a higher energy state , when falling back to the lower energy state , the emitted energy will react with cellular oxygen or / other cellular components to produce reactive species such as singlet oxygen and free radicals , the site of action for the cytotoxic species produced during lethal photo-sensitization has been investigated in a number of studies , the three main sites are cell membrane , the nucleus and organelles , increasing ion permeability and loss of fluidity is a result of the transfer of triplet state photosensitizer energy to molecular oxygen , forming the singlet oxygen which is the main bactericidal species , and cause lipid per oxidation , which is highly detrimental to cell membrane structure and function and cause cell death (27,28). A previous study by Lipovsky (29) found when used white light, caused a reduction of the colony count of *Escherichia coli, S. aureus* and *S. marcescens,* respectively. The phototoxic effect was found to involve induction of ROS production by the bacteria. Visible light at high intensity can kill bacteria in infected wounds. Infected wounds with intense visible light, prior to low intensity illumination

for stimulating wound closure, reduced infection and promoted with healing.

Study by Hashmi *et al.* (30). Found that low-level of laser therapy (LLLT) has become an increasingly mainstream modality, especially in the areas of physical medicine and rehabilitation. At first used mainly for wound healing and pain relief, the medical applications of LLLT have broadened to include diseases such as stroke, myocardial infarction, and degenerative or traumatic brain disorders. The mechanisms of LLLT that operate both on a cellular and a tissue level. Mitochondria are thought to be the principal photoreceptors, and increased adenosine triphosphate, reactive oxygen species, intracellular calcium, and release of nitric oxide are the initial events. Activation of transcription factors then leads to expression of many protective, anti-apoptotic, anti-oxidant, and pro-proliferation gene products. Animal studies and human clinical trials of LLLT for indications with relevance to neurology, such as stroke, traumatic brain injury, degenerative brain disease, spinal cord injury, and peripheral nerve regeneration, will be covered. Study by Lee *et al*. (31) investigated the photobiomodulation effects of 1072 nm infrared light on the natural immune response involved in anti-bacterial and wound healing processes. Thirty mice infected with MRSA on the skin were divided into two groups. The experimental group was treated with 1072 nm infrared light , Serial changes of the mRNA levels of TLR2, IL-1β, TNF-α, IL-6, iNOS, MCP-1, TGF-β, bFGF and VEGF were studied by real time RT-PCR and those of the expression level of VEGF, bFGF, TGF-β and NF-κB by immunohistochemistry. infrared light (1072 nm) had a photobiomodulation effect which resulted in an enhanced biological immune response to the bacterial infection by MRSA and also increased the expression of VEGF to a significant level. AL-Obaidi *et al*. (32) concluded that laser photosensitiser combination had greater efficacy for killing *Leismania* promastigote stage in vitro than laser light alone. There are two main mechanisms of lasers in medical applications: The first is bio-stimulation mechanism. Stimulus is a concept on the biological functions. According to the biological function, autonomic nerve reflex, Pavlov's molecular biology and the principle of theory, can think that the weak interaction between laser and organism, laser is a source of stimulation, and the organism has special feel for various stimuli (33). *In vivo* impulse caused by receptor for stimulation, afferent nerve endings, reach the reactor reaction. Response is excited or inhibited. The relative dose depends on the application of laser. Part of the proper dose of laser energy in certain organisms, as appropriate amount of stimulation answer in response to this, at the molecular level is to adjust the synthesis of protein and nucleic acid, DNA replication, regulation of enzyme function at the cellular level is the mobilization of compensation, nutrition, repair, and other immune defense mechanisms to eliminate the pathological process (34). The mechanism of laser biostimulation is a reasoning hypothesis. In foreign countries, the weak laser bio-stimulation mechanism theory is inconsistent. A biological electric field hypothesis, light regulation system hypothesis, cell membrane receptor hypothesis and the hypothesis of the

four polarization stimulation is not consistent with the hypothesis. This shows that the theory is not mature and need to be further discussed (35). The second mechanism is thermal effect. The thermal effect is the main factor of biological effect. It plays a role in all of the laser irradiation. High power semiconductor laser is mainly thermal effects of its application. Laser in biological tissue, tissue molecular absorption photon energy, the vibration and rotation increased, in the macro performance of the local irradiation becomes hot, high temperature. Because there exist such as melanin, hemoglobin, carotenoids and other pigments in the tissue cells, can increase the light absorption, so that the heat effect of laser is more significant (36).The interaction between laser and biological is a complicated process determined by various factors. The laser parameters such as wavelength, power, energy, coherence, polarization mode, laser modes have different effects on the biological tissue. The biological tissue properties such as density, elasticity, thermal conductivity, heat capacity, thermal diffusivity, reflectivity, absorption rate, pigment, moisture content, uniformity and hierarchical structure, also will make a different reflection of laser. In recent years, research on laser and biological interaction gradually, mainly discusses the absorption and scattering of laser tissue from theory and experiment, determine the temperature distribution and organization within the organization within the laser energy (12).

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