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Effects of some physical agents on *Staphylococcus epidermidis* and *Leishmania tropica:* An *In vitro* study

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Abstract

This work evaluated the effect of Alpha, Gamma irradiation and Nd:YAG, He-Ne laser on *Staphylococcus epidermidis* and *Leishmania tropica in vitro*. The experiment included five replicate of *S. epidermidis* , *L. tropica in vitro* exposed to effect of Alpha , Gamma irradiation by ^{241}Am isotopes , in two doses γ = 0.31993 MsV (Milli Sievert) for 3 hr, and in dose α= 1.4157 MsV for 3hr , Americium isotopes give two type of decay Alpha and Gamma Rays. Effect of Nd:YAG laser in 1000 pulse between each pulse 6 sec, in wavelength 1.06 nm ; also He-Ne laser in 5, 10 , 20 , 30 minute , with wavelength 632.8nm. The effect of Nd:YAG, He-Ne laser and Alpha, Gamma irradiation on the viability of *S. epidermidis* , *L. tropica in vitro* was determined by using the specific equation compared with Control and determined by using colorimetric MTT assay respectively , the number of viable cells of *S. epidermidis* , *L. tropica* was fewer than control (without exposure to laser and irradiation). Nd:YAG laser, He-Ne laser and Alpha , Gamma irradiation was efficient to kill *S. epidermidis* and *L. tropica* .

Keywords: Nd:YAG, He-Ne, Americium isotopes, *Staphylococcus epidermidis, Leishmania tropica* .

تأثير بعض العوامل الفيزيائية على المكورات الجلدية و واللشمانيا الجلدية: دراسة في المختبر

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الخلاصة

تم القيام بهذا العمل بتأثير أشعة كاما وألفا وليزر أندياك ، ليزر الهيليوم–نيون على بكتريا المكورات الجلدية واللشمانيا الجلدية في المختبر. التجربة تضمنت خمس مكررات معرضة الى أشعة ألفا وكاما من قبل المصدر المشع أمريشيوم في جرعتين كاما = 0,31993 ملي سيفرت لمدة ثلاث ساعات وجرعة ألفا =1,4157 ملي سيفرت لمدة ثلاث ساعات ، المصدر المشع الأمريشيوم يعطي نوعين من الأشعاع أشعة ألفا وكاما. تأثير ليزر أندياك في 1000 نبضة بين كل نبضة ونبضة 6 ثانية في طول موجي 1.06 نانوميتر وأيضا التعريض الى ليزر هيليوم– نيون في(5، 10، 15 ،20) دقيقة بطول موجي 632.8 نانوميتر . أن تأثير ليزر أندياك وليزر هيليوم –نيون وأشعة ألفا وكاما على حيوية بكتريا المكورات الجلدية واللشمانيا الجلدية في المختبر حددت بواسطة معادلة خاصة بالمقارنة مع السيطرة السالبة (بدون التعريض الى الاشعاع والليزر) وحددت بأستخدام فحص خاص على التوالي، عدد الخلايا الحية لبكتريا المكورات الجلدية واللشمانيا الجلدية كانت أقل من السيطرة السالبة ، ليزر أندياك وهيليوم– نيون وأشعة كاما وألفا كانت كفوءة في قتل البكتريا الجلدية واللشمانيا الجلدية .

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Introduction

S. epidermidis is a Gram-positive bacteria, they are most likely harmless. It is part of the normal human flora, skin flora [1]. But they are hospital pathogens, especially those with medical devices, [2]. Facultative anaerobic bacteria, nosocomial infections [3]. These infections are generally hospital-acquired[4].

The most common infections on catheters and implants [5]. For people with catheters or other surgical implants due to form biofilms grown on devices [6]. It is found inside acne vulgaris pores [7]. The drug of choice is often vancomycin. *S. epidermidis* increase causes a problem since is resistant to methicillin and all penicillins, penems, carbapanems, and cephalosporins which are commonly used antibiotics [8].

Leishmania are obligate intracellular parasites, they are the causative agents of group of diseases called leishmaniasis [9].

Cutaneous leishmaniasis (CL) is affect the skin and mucous membranes, mucosal leishmaniasis the parasite may spread to the mucous membranes of the nose, throat and mouth[10]. *Leishmania major, L. tropica, L. braziliensis are* the most common and least fatal form of the disease, identified by ulcerative skin lesions [11].

Different types of rays like γ -ray, UV light and laser effect on microorganisms.There are many application rays it's used in vaccination and treatment [12]. Laser, UV and gamma rays have different effects on material [13].

Ionizing radiation was used for the treatment of many types of infections. Radiation used to physical sterilization, as a cold process, widely used for the sterilization of health care products [14].

The exposure of microbial cells to ionizing radiation leading to stress to the cells which will tends to disturb the organization of Nucleic acids DNA, breaks in DNA will cause disrupt function of the molecule in several ways[15].

Gamma rays is an electromagnetic radiation is put out as a result of moving the nucleus from the excited status to the ground state directly or in stages to move to a state of less than signal down to the ground status as a result of any other nuclear process kanavat alpha, beta or another nuclear reaction to get rid of stimulation energy[16].

Laser is an acronym of light extension by the catalyzed emission of radiation and is equipage that converts electrical energy into light energy, there are three basic types of effect on living tissue photothermat , photochemical , and photo-caustic. In medicine, laser is used as therapeutic agents used in ophthalmology, dermatology, gynecology and surgery [17].

The laser is one of the most Bactericidal, which have a fatal effect on Gram negative and Gram positive bacterial species, effect on *Pseudomonas aeruginosa* and other bacteria [18]. When laser radiation encounters matter the scattered photons will be absorbed [19].

He-Ne (Helium-Neon) is the first source of coherent light, a photo biologic, a bio-stimulation treatment for the stomach, the twelve and duodenal ulcers[20]. He-Ne laser has locative feature of 632.8 nm wavelength, good directivity, high intensity, good monochromatic and cohesion is a changing low-level laser. The low-level laser has some biology effects such as cell liveliness, phagocytosis, its usage for the treatment of digestive disease[21].

Nd:YAG lasers emit light at 1064 nm used widely for thermotherapy, in which benign or malignant lesions in diverse organs[22].,break down primary and secondary malignant liver lesions[23,24]. They are also used to decrease benign thyroid nodules [25]. Also used in in oncology. Nd:YAG lasers can be used to take off skin cancers[22].

The aim of this study to research the efficiency of Alpha, Gamma irradiation and Nd: YAG, He-Ne laser on the viability of on *S. epidermidis, L. tropica in vitro*.

Materials and methods

Bacteria and Parasite cultivation and exposure

Five isolates of *S.epidermidis* and *L. tropica* were obtained from Al mustansyriah University/college of science 2017-2018. The cultivation was done in accordance with Tramps *et al.* with some alterations. *S. epidermidis* were cultivated in Nutrient agar and *L. tropica* cultivated in M199 media at 37°C and 25° C for 18-24hr. and five days respectively, then centrifuged (5000 rpm for 10 minutes). The pellet was suspended in 150 ml of sterile normal saline, then 1 ml of solution was exposure to Alpha, Gamma irradiation ^{231}Am Americium isotopes in dose **γ** = 0.31993 MsV for 3 hr,

and in dose α= 1.4157 MsV for 3hr, in compare with control (without exposition), and injected in Nutrient agar and M199 media for bacteria and parasite.

Also used 1000 pulse of Nd:YAG .energy per pulse (energy density or power density) is 700 mJ . pulse duration is 10 ns , With Pulsed peak power 70 m J /ns. repetation rate of 1 Hz and effective beam diameter of 4.8nm with wavelenghth 1.06 nm[26].

Also used He-Ne laser for (5, 10, 20, 30) minute with wavelength 632.8 nm ,mode of laser is C.W laser , output power 1mW , pulse duration is C.W laser , with power density 0.1624 mW/mm^2 , spot size of exposure to laser light is 6.154 mm^2 .

Bacteria and Parasites viability determination

In vitro S. epidermidis, L. tropica viability was determined by using count on plate and by using MTT assay respectively.

Viability *S. epidermidis* was determined by using the equation below:

Viability of *L. tropica* was determined by using the equation below:

Percentage of Killing = Control – treated *10 Control

Viable cells (%) = (AT-AB) / (AC-AB) ×100

Where AC, AT and AB is the absorbance of the not treated, treated samples and blank respectively [27].

Statistical Analysis of Bacteria and parasite

The Statistical Analysis System- SAS program [28].was used to analyze the effect of different agents in studied indicators . Chi-square test was used to significant compare between them.

Results and discussion

The effect of Alpha, Gamma, irradiation and Nd-YAG, He-Ne laser and directly on *S.epidermidis* , *L. tropica* viability.

The deadly effect of ionizing irradiation on microorganisms, as standardize by the absence cells of colony-forming ability in Nutrient medium and in other medium[29].

After *S.epidermidis* and *L. tropica* been exposed to Alpha, Gamma rays of ^{241}Am isotopes in dose dose γ = 0.31993 MsV for 3 hr. and in dose α= 1.4157 MsV for 3hr. and to 1000 pulse of Nd:YAG laser with wavelength 1.06 nm , also exposition to He-Ne laser for (5, 10, 20, 30) minute with wavelength 632.8 nm and the viability of these cells determined using Count on plate , calculated from previous equation with Control (without exposure to laser and irradiation) which was shown in Tables-(1, 2 and 3). and by using MTT assay to determined viable cell of *L.tropica* which was shown in Tables-(4,5 and 6), thus cell survival was lowering with prolonged exposition to Alpha, Gamma irradiation and Nd-YAG laser and He-Ne laser .

Several scientists suggested the mechanics of radiation idea 'radiotoxins' that are the toxic substances created in the irradiated cells accountable for lethal effect. Others suggest that radiation was immediately deleterious the cellular membranes, on enzymes, on energy metabolism, on the cytoplasmic membrane[29].

All method was used Alpha, Gamma irradiation, Nd:YAG laser , He-Ne laser was efficient to kill *S. epidermidis* and *L. tropica in vitro*, these results help use Alpha, Gamma irradiation and laser in the treatment of many infections in humans.

Table 1-The percentage of killing and Number of viable cells of *S.epidermidis* (mean of 5 isolates) after exposure to Alpha and Gamma irradiation by Americium isotopes.

	Isotopes	Time of exposure	Type of decay	Dose (MsV)	No. of Viable cells	Precentage of Killing %	p-value
1	^{241} Am	3hr	γ	0.31993	29	90.3%	0.001**
2	^{241} Am	3hr	α	1.4157	50	83.3%	0.001**
Control = 300							

Table 2-The percentage of killing and Number viable cells of *S.epidermidis* after exposure to Nd-YAG laser

	percentage of killing and NO. of viable cells exposed to Nd-YAG laser		
	1000 pulse precentage of killing	1000 pulse (No. viable)	p-value
1	83.3%	50	0.0001**
2	91 %	27	0.0001**
3	87.6 %	37	0.0001**
4	95 %	15	0.0001**
5	90 %	30	0.0001**
Control =300			

There are so used to decrease benign thyroid nodules[30], and to break down primary and secondary malignant liver damage[31,32].

The fatal effect of ionizing radiance on microorganisms by cell damage 'radio-toxins' that toxic

A previous study was used low dose of Gamma radiance which was an efficient method for decreasing and killing *S. aureus* [33].

Table 3-The percentage of killing and No. viable cells of *S.epidermidis* after exposure to He-Ne laser.

	Percentage of cell viabe and percentage of cell killing exposure to He-Ne laser			
1	**5min**		**10min**	
	NO. Viable	Precentage of killing	NO. Viable Cell	Precentage of killing
	13	95.6 %	7	97.6 %
	0.0001**		0.0001 **	
2	**20min**		**30min**	
	NO.of Viable cell	Precentage of killing	NO. Viable **Cell**	Precentage of killing
	1	99.6 %	0	100 %
	0.0001**		0.0001 **	
Control = 300				

Radiance variance not only by their content (electrons, protons, neutrons) but so by their energy. high-LET radiance are more devastating to biological substance than low-LET radiance-such as X and gamma radiation of DNA damage of microorganisms causative by intense ionizations from high-LET radiance is more hard to repair than the prevalent DNA damage give rise to by the little ionizations from low-LET radiance [34].

Ray-induced ionizations perhaps work immediately on the cellular content molecules or in a bad way on water molecules, give rise to water-derived radicals. Radicals interact with close molecules in a much short time, give rise to in fracturing of chemical bonds or of the influenced molecules. The main action in cells is DNA break up[35].

Table 4-The percentage of killing and viable cells of *L. tropica* (mean of 5 isolates) after exposure to Alpha, Gamma irradiation by Americium isotopes

Absorbance	Isotopes	Time of exposure	Type of decay	Dose (MsV)	killing %	Viable %	p-value
490nm	**^{241} Am**	**3hr**	**γ**	0.31993	86.4%	13.6%	0.001**
490nm	**^{241} Am**	**3hr**	**α**	1.4157	97.2 %	2.8 %	0.001**

The prime involvement of cell death show to be the influence among near-infrared spectrum laser light and the bacterial micro-medium [36].

A previous study by Luan[37].Showed that the antimicrobial effectiveness of laser was believed as a secure co adjutant in nonsurgical therapy of gingivitis, diminish the symptoms of inflammation and microbial contagion wanting any hurtful effects on close periodontal tissues.

Nd:YAG laser (bactericidal) on a suspension of *Escherichia coli* was shown that a temperature rise up to 50º C after the use of laser with a power productivity of 100 W for 23 second put to death bacteria [38].

Table 5-The percentage of killing and viable cells *L. tropica* next exposition to Nd-YAG laser

	percentage of cell killing exposed Percentage of cell viability and to Nd-YAG laser		
	1000 pulse Percentage of killing)	1000 pulse Precentage of viable cells	p-value
1	91 %	9 %	0.0001**
2	80 %	20%	0.0001**
3	97.6 %	2.4 %	0.0001**
4	91 %	9 %	0.0001**
5	95 %	5 %	0.0001**

Table 6-The percentage of killing and viable cells of *L. tropica* (mean of 5 isolates) after exposure to He- Ne laser

	Percentage of cell viability and percentage of cell killing exposed to He-Ne laser			
490nm	**5min**		**10min**	
	78.12 %	21.88 %	80.4 %	19.6 %
	0.0001**		0.0001 **	
	20min		**30min**	
	Killed	**Viable**	**Killed**	**Viable**
490nm	89.22 %	10.78 %	94.33%	5.67%
	0.0001**		0.0001 **	

Previous study done by Mutinga [39]. successfully remedy three status of acute cutaneous leishmaniasis by collective Ultra violet light and infrared treatment

Irradiation effect to removal of *S. aureus* so as to reason much infection in skin of human infections ability prevalence over reach to pus of an infected wound, skin-to-skin contact with an infective individual [40]

A light of down –power laser with wavelength , the released energy will interact with cell oxygen or / other cell components to make interactive species such as singlet oxygen and open radicals, the three major sites are cell membrane , the nucleus and organelles causes rising ion permeability , loss of liquidity , cause cell death [41].

Other study by Gautam [42].Find irradiation sensitivity of *K. pneumoniae* MTCC 109 was efficient of different food samples 1.5 kGy dose result elimination of *K. pneumoniae* from food samples (sprouts, poultry and fish) samples.

He-Ne, Low intensity radiation of (wavelength 632.8 nm) was utilized successfully for treatment alimentary ulcers and slow wounds of various etiology while classic drug curing as active applied for treatment regional lesions and systemic [43].

Nd-YAG laser has great energy and penetrates deep into tissues [44].Many previous studies the thermic action of Nd:YAG laser to bacteria. The bactericidal effect of a height-power [45].Nd-YAG laser has good therapeutic effect and smooth postoperative period with no significant pain and discomfort, making it an appropriate solution in complex treatment of the disease [46].

The study by Moon [47].was 1064-nm long-pulsed Nd:YAG laser used as a safe and effective treatment modality of onychomycosis, the 1064nm Nd:YAG PinPointe Foot Laser as an effective alternative therapy to the typical oral and topical medications. it is quick high revenues and easy to incorporate into a practice, with high patient satisfaction .

The present study suggests the possibility of the experience of these kinds of transactions medically on the several infections caused by *S.epidermidis* and *L.tropica* infection, which may give good results as in those caused by many bacteria and parasite.

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