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The Effect of Nd:YAG, Semiconductor, He-Ne laser and Beta, Gamma irradiation on *Leishmania tropica* Promastigotes

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Abstract: This work evaluated the effect of Nd:YAG, Semiconductor, He-Ne laser and Beta, Gamma irradiation on *Leishmania tropica* promastigotes *in vitro*. The experiment included a control and tetra-plicate of *L. tropica* promastigotes exposed to Nd:YAG laser in 500 pulse between each pulse 6 sec, in wavelength 1060 A°; the effect of Semiconductor laser in (5, 10, 20, 30) minute , with wavelength 532 nm; also use He-Ne laser in (5, 10 , 20 , 30) minute , with wavelength 6328 A°; and effect of Beta and Gamma irradiation by ^{137}Cs isotopes , in two doses (1.105607×10^{-7} Gy , for 2hr, and in dose 1.650992×10^{-7} Gy for 3hr), cesium isotopes that give two type of decay Gamma and Beta Rays. The effect of Nd:YAG, Semiconductor, He-Ne laser and Beta, Gamma irradiation on the viability of *L. tropica* promastigote was determined using the colorimetric MTT assay, the number of viable cells of *L. tropica* was fewer than control (without exposure to laser and irradiation). Nd:YAG laser, Semiconductor laser, He-Ne laser and Beta , Gamma irradiation was efficient in killing *L. tropica* promastigotes *in vitro*, and the remain of *L. tropica* after exposure to laser and irradiation was devoid flagellum and may affect the capability of infection.

Keywords: *Leishmania* attenuation, cutaneous leishmaniasis, laser types, laser wavelengths

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Introduction

Leishmaniasis is a clinically heterogeneous group of diseases, caused by protozoa of the genus *Leishmania*. Disease can range from a solitary, spontaneous healing ulcer (Cutaneous leishmaniasis), to generalized involvement with visceral leishmaniasis (1). Cutaneous leishmaniasis (CL) is the most common and least fatal form of the disease, identified by ulcerative skin lesions, and it is caused by *Leishmania major, L. tropica, L. aethiopica, L. mexicana, L. braziliensis, L. guyanensis, L. panamensis, L. peruviana,* and *L. amazonensis.* Almost two-thirds cases of CL are reported from six countries: Afghanistan, Algeria, Brazil, Colombia, Iran and the Syrian Arab Republic (2). Previous studies have shown the effect of different types of rays like γ -ray, UV light and laser on *Leishmania* parasites. There are many sites for the application rays for example it's used in vaccination and treatment (3). Laser is an acronym of light amplification by the stimulated emission of radiation and is advice that converts electrical energy into light energy, when laser beam encounters matter; photons are either reflected transmitted, scattered or absorbed. A portion of the beam might be reflected

by being back scattered , transmitted without effect on the tissue or absorbed, absorption of laser lights depends on many parameters like wavelength and the types of tissues , there are three basic types of effect on living tissue such as photothermat , photochemical , and photo-caustic. In several branches of medicine, laser is used as therapeutic agents such as in ophthalmology, dermatology, gynecology and surgery (4). Type of the laser is named with its wavelength on the radiation spectrum (810 nm) or its active lasing medium (diode laser). Active lasing mediums can be: gas, liquid, solid, and semiconductor or biologic materials. Solid state lasing mediums are commonly in glass or crystal form. Active lasing material carried by a host material. Most common host material is yttrium aluminum-garnet (YAG) combination. Several active lasing materials like neodymium (Nd:YAG) neodymium-doped yttrium aluminum garnet. Neodymium ions in various types of ionic crystals, and also in glasses, act as a laser gain medium, typically emitting 1064 nm light from a particular atomic transition in the neodymium ion, after being "pumped" into excitation from an external source (5). Nd:YAG lasers emitting light at 1064 nm have been the most widely used laser for laser-induced thermotherapy, in which benign or malignant lesions in various organs are ablated by the beam. In oncology, Nd:YAG lasers can be used to remove skin cancers(6). They are also used to reduce benign thyroid nodules (7), and to destroy primary and secondary malignant liver lesions (8, 9). 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 (10). 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 (11). He-Ne laser was the first widely accessible source of coherent light, has a photo-biological nature, has equal efficiency of non-coherent and laser light the bio-stimulation treatment of stomach and duodenal ulcers (12). Beta rays is electrons or neutrons (positively charged electron), it possesses high speed produced from the nucleus as a result of the disintegration of the proton or neutron and accompanying emitted particle known as the neutrino or anti-neutrino, respectively, Gamma rays is a electromagnetic radiation is issued as a result of moving the nucleus from the excited state to the ground state directly or in stages to move to a state of less than signal down to the ground state as a result of any other nuclear process kanavat alpha , beta or another nuclear reaction to get rid of excitation energy (13).Gamma irradiation is electro-magnetic radiation of short wavelength emitted by radioactive isotopes as the unstable nucleus breaks up and decays to reach a stable form, It is widely used for sterilization of medical devices, food preservation and processing of tissue allografts and blood components, obviating the need for high temperatures that can be damaging to such products (14). DNA is the principal cellular target governing loss of viability after exposure to gamma irradiation (15). 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 (16). 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 (17). The aim of this study was to investigate the effectiveness of Nd: YAG, Semiconductor, He-Ne laser and Beta, Gamma irradiation on the viability of *Leishmania tropica* promastigotes.

Materials and methods

Parasite cultivation and exposure

Four isolates of *Leishmania tropica* parasites were obtained from college of science-university of Baghdad. Parasite cultivation was done according to Tramps *et al.* (14) with some modifications as follows:

L. tropica promastigotes were cultivated in M199 media at 25° C for five days to reach the stationary-phase culture, then culture was centrifuged (5000 rpm for 10 minutes). The pellet was suspended in 150 ml of sterile normal saline, then 1 ml of this solution was exposed to 500 pulse of Nd:YAG with mode of laser is pulse laser . Energy per pulse (energy density or power density) is 700 mJ. pulse duration is 10 ns , repetation rate of 1 Hz and effective beam diameter of 4.8nm .

$$\text{Pulsed peak power} = \frac{\text{Pulse Energy}}{\text{Pulse duration}} = \frac{700 \text{ m J}}{10 \text{ ns}} = 70 \text{ m J /ns}$$

Used in the device 500 V to operate the machine at ħυ =532 (freguency doubling), 500 was used for pulse irradiation. Also used Semiconductor for (5, 10, 20, 30) minute with wavelength 532 nm, (continuous laser), output for S.C. laser (ħυ =532 +-10) nm . the output power = < 200 mW. for S.C. laser ħυ = (650+-10) nm , the output power =< 100 mW . energy density or power density for (ħυ =532 +-10) nm , power density is 200 mW /cm^2 .but in S.C. laser ħυ = (650+-10) nm ,power density is 100 mW/ cm^2 . .spot size of exposure to laser light =1 cm^2. And used He-Ne laser for (5, 10, 20, 30) minute with wavelength 6328 A° ,mode of laser is C.W laser , output power 1mW , pulse duration is C.W laser , with energy density or power density 0.1624 mW/mm^2 , spot size of exposure to laser light is 6.154 mm^2 , or to Beta, Gamma irradiation of ^{137}CS isotopes in dose 1.105607×10^{-7} Gy for 2hr, and 1.650992×10^{-7} Gy for 3hr, in comparison to control group (without exposure), and inoculated in M199 media.

Parasites viability determination

In vitro parasites viability was determined by using MTT assay.

MTT assay principal and protocol

MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide; thiazolyl blue] is a water soluble tetrazolium salt yielding a yellowish solution. Dissolved MTT is converted to

an insoluble purple formazan by cleavage of the tetrazolium ring by dehydrogenase enzymes (18). This water insoluble formazan can be solubilized using Dimethyl sulfoxide (DMSO), and the dissolved material is measured spectrophoto-metrically yielding absorbance as a function of concentration of converted dye (19).

Relative numbers of live cells were determined based on the optical absorbance of the treated and untreated samples and blank wells using the formula mentioned below. *L. tropica* promatigotes was prepared in 96-well plates in a final volume of 100µl/well and incubated at 25˚C for three days. Ten µl of MTT solution was added per well and then the plate was incubated for 4 hr at 25˚C. The media was removed and 100µl of DMSO solution was added in order to solubilize the formazan crystals. The plate was stirring gently then, left for 15 minutes. Absorbance was recorded at 490 nm by micro-plate reader and viability determined using the formula:
Viable cells (%) = (AT-AB) / (AC-AB) ×100 Where AC, AT and AB is the absorbance of the untreated, treated samples and blank respectively (20).

Statistical Analysis

The Statistical Analysis System-SAS (21). Program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between.

Results and discussion

This study aims to prove the effect of Nd-YAG, Semiconductor, He-Ne laser and Beta, Gamma irradiation directly on the promastigotes viability, so it can be test the usability of parasite attenuation for use in immunizing laboratory animals later or in direct cutaneous leishmaniasis treatment. After the exposure of *L. tropica* promastigotes to (500 pulse) of Nd:YAG laser, or semiconductor for 5, 10, 20, 30 minutes with wavelength 532 nm, or He-Ne laser for 5, 10, 20, 30 minutes with wavelength 6328 A° and to Beta, Gamma irradiation of ^{137}CS isotopes in dose 1.105607×10^{-7} Gy for 2hr and 1.650992×10^{-7} Gy for 3hr, the viability of these cells determined using MTT assay which was shown in Tables 1,2,3, thus cell viability was decreased with long exposure to Nd-YAG laser, Semiconductor, He-Ne laser and Beta ,Gamma irradiation . Each Nd:YAG laser , Semiconductor laser , He-Ne laser and Beta ,and Gamma irradiation was efficient in killing *L. tropica* promastigotes, these results help may be in the use of laser and Gamma , Beta irradiation in the treatment of cutaneous leishmaniasis infections in humans.

Table (1): The percentage of viable *L. tropica* promastigotes after exposure to Nd-YAG laser.

	Percentage of cell viability and percentage of cell killing exposed to Nd-YAG laser		
	500 pulse(killing)	500 pulse (viable)	p-value
1	97.29 %	2.7 %	0.0001**
2	91.89%	8.1%	0.0001**
3	100%	0%	0.0001**
4	89.18%	10.8%	0.0001**
Control.= 0.1 , blank = 0.063 , wavelength = 490 nm			

Table (2): The percentage of viable *L. tropica* promastigotes (mean of 4 isolates) after exposure to Semiconductor laser and He- Ne laser.

Absorbance	Percentage of cell viability and percentage of cell killing exposed to semiconductor laser			
	5 min		**10 min**	
	killed	**Viable**	**killed**	**viable**
490nm	64.86 %	35.14 %	81.08 %	18.92%
	0.0027 **		0.0001 **	
	20min		**30min**	
	Killed	**viable**	**Killed**	**Viable**
490nm	94.59%	5.41%	100%	0%
	0.0001 **		0.0001 **	
Control.= 0.1 , blank = 0.063 , wavelength = 490 nm				
490nm	**Percentage of cell viability and percentage of cell killing exposed to He-Ne laser**			
	5min		**10min**	
	89.18%	10.82%	91.89%	8.11%
	0.0001**		0.0001 **	
	20min		**30min**	
	Killed	**Viable**	**Killed**	**Viable**
490nm	94.59%	5.41%	97.29%	2.71%
	0.0001**		0.0001 **	
Control.= 0.1 , blank = 0.063 , wavelength = 490 nm				

****p*<0.01 level.**

Table (3): The percentage of viable *L. tropica* promastigotes (mean of 4 isolates) after exposure to Beta and Gamma irradiation.

Absorbance	Isotopes	Time of exposure	Type of decay	Dose (Gy)	Viable cells%	Killing cell%	p-value
490nm	^{137} CS	2hr	ɣ , β	1.105607×10^{-7}	72.98 %	27.02 %	0.001**
490nm	^{137} CS	3hr	ɣ , β	1.650992×10^{-7}	0 %	100%	0.001**
Control.= 0.1 , blank = 0.063 , wavelength = 490 nm							

****p*<0.01level.**

Also, it was clear that each of Nd-YAG laser, Semiconductor laser, He-Ne laser, and Beta and Gamma irradiation affect the parasite morphology and motility as shown in Figures 1, 2 which revealed the parasites devoid their flagella, which perhaps may affect negatively their ability to penetrate and infect host cells.

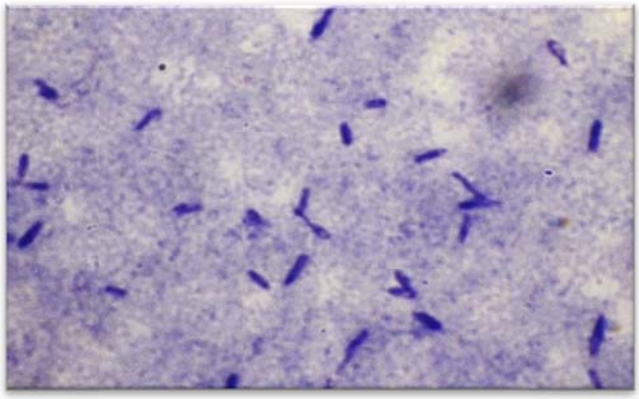

Figure (1): *L. tropica* promastigotes after exposure to Nd:YAG laser

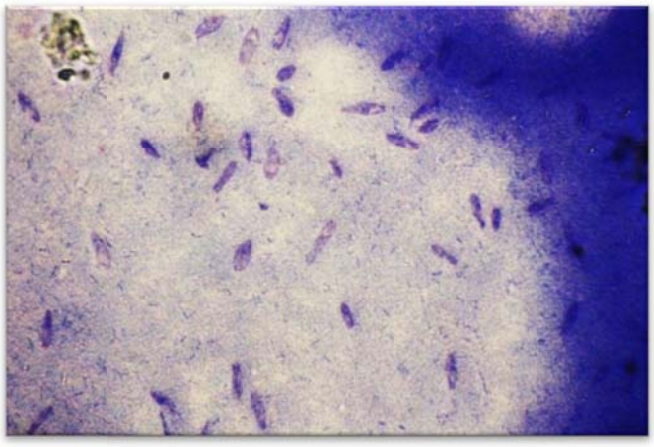

Figure (2): *L. tropica* promastigotes after exposure to Semiconductor laser

Many previous studies focused on the thermal effect of Nd:YAG laser on the bacteria, but no study shows on its effect on *Leishmania*. The bactericidal action of a high-power Nd:YAG laser on a suspension of *Escherichia coli* was shown that the temperature rise up to 50º C after the use of laser with a power output of 100 W for 23 second (22).

A previous study done by Pirnat *et al*.(23) 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 *Leishmania.*

Nd-YAG laser has great energy and penetrates deep into tissues. In medicine it is used for ablation of oral leukoplakia with perfect results, thermal effect and pain during the procedure, requiring anesthesia, should be believed as negative aspects of the treatment with this type of laser (24), while, Lalabonova *etal*. (25) showed that 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.

Also, the 1064-nm long-pulsed Nd:YAG laser used as a safe and effective treatment modality of onychomycosis, these data suggests that the 1064nm Nd:YAG PinPointe FootLaser should be considered as an effective alternative therapy to the typical oral and topical medications. Furthermore, it is quick high revenues and easy to incorporate into a practice, with high patient satisfaction (26).

Asnaashari and Safavi, (27), used various laser wavelengths (Diode and Nd:YAG lasers) in combination with sodium hypochlorite in appropriate concentrations in success root canal therapy. For the treatment of cutaneous leishmaniasis, Asilian *et al*. (28) obtained effective results with CO_2 laser for both wet and dry types of cutaneous leishmaniasis. Also Al-Muslet and Khalid, (29) showed that 92.3 % of treated patient revealed excellent response to the low level of laser therapy. It was clear that *Leishmania* parasites are thermo-sensitive, *in vitro* study, *L. tropica* can multiplied at 35C° and was completely eliminated over than 37C°, and therefore both heat and cold treatment have been tried. In Iraq infrared heat was used to raise the temperature of cutaneous leishmaniasis lesions to 55C ° for 5 minutes, and all lesions healed in 5 to 6 weeks (4). Previous study successfully treated three cases of acute cutaneous leishmaniasis by combined Ultra violet light and infrared therapy (30). Low intensity radiation of He-Ne (wavelength 632.8 nm) was previously used successfully for treating trophic ulcers and indolent wounds of diverse etiology when traditional drug treatment has not been as effective used for treating not only local lesions but very often also "systemic" (12). Many hypotheses have been proposed and tested on the lethal effect of ionizing radiation on microorganisms, regarding the mechanism of cell damage by radiation. Some scientists proposed the mechanism thought ‘radio-toxins’ 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 (31). Gamma rays cause damage at a cellular level and are penetrating **VG**. However, they are less ionizing than Alpha or Beta particles, which are less penetrating (32), therefore by mechanisms of Gamma and Beta irradiation can be elimination of *S. aureus* that causes many infection to skin of human, particularly infections which can spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroys tissues, and contact with objects such as towels, sheets, clothing, or athletic equipment used by an infected person. Deeply penetrating *S. aureus* infections can be severe (33). Lamb *et al.,* (34) was used low dose of Gamma irradiation which was an effective method for reducing and killing *S. aureus*. The present study suggests the possibility of the experience of these kinds of transactions medically on the cutaneous lesions caused by cutaneous leishmaniasis, which may give good results as in those caused by bacteria.

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