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The Effect of Semiconductor , He-Ne laser and Beta , Gamma irradiation on *Leishmania donovani* promastigote

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Abstract

This work evaluated the effect of Semiconductor , He-Ne laser and Beta , Gamma irradiation on *Leishmania donovani* promastigotes . The experiment included a control and tetraplicate of *L. donovani* promastigotes exposed to Semiconductor laser in (5, 10, 20, 30) minute , with wavelenghth 532 nm ; also use He-Ne laser in (5, 10 , 20 , 30) minute , with wavelenghth 6328 A° ; and effect of Beta and Gamma irradiation by ^{137}Cs isotopes , in dose $1.776*10^{-4}$ **µ**sV of Beta ray (energy 0.514 MeV) and exposure to Gamma ray(energy 0.662 MeV) in dose 96.950 **µ**sV in 2hr. , cesium isotopes (^{137} Cs) that give two type of decay Gamma and Beta Rays . and exposure to ^{90}Sr that give one type of decay is Beta rays (energy 0.198 MeV) in dose 63.100 **µ**sV . The effect of Semiconductor , He-Ne laser and Beta, Gamma irradiation on the viability of *L. donovani* promastigote is count and percentage of killing count, the number of viable cells of *L. donovani* is fewer than control (without exposure to laser and irradiation) , but percentage of killing is higher than control . Semiconductor laser , He-Ne laser and Beta , Gamma irradiation is efficient to killing *L. donovani* , and the remain of *L. donovani* after exposure to laser and irradiation is devoid flagellum and not cause reinfection but attenuated . Semiconductor , He-Ne laser and Beta , Gamma irradiation is effective to killing *L. donovani* .

Keywords: Leishmania donovani , Vesiral leishmaniasis, laser types, laser wavelengths, Irradiation .

Introduction

Protozoan parasites of the genus *Leishmania* cause a spectrum of diseases in humans, ranging from self-healing ulcers to potentially fatal visceral leishmaniasis, which affect millions of people worldwide (Matte, 2010).

Leishmaniasis, is caused by obligate intra-macrophage protozoa, is endemic in large areas of the tropics, subtropics and the Mediterranean basin . it is caused by more than 20 leishmanial species and is transmitted to humans by ~30 different species of phlebotomine sand flies (Pearson and Sausa , 1996).

Visceral leishmaniasis (VL) is one type of leishmanial infections that remains asymptomatic or subclinical in many cases or can follow an acute or chronic course , The clinical symptoms are characterized by prolonged and irregular fever often associated with rigor and chills, splenomegaly lymphadenopathy, hepatomegaly, pancytopenia progressive anaemia and weight loss, It is always fatal if left untreated (Singh *et al.,* 2006) .

Among several drugs used in the treatment of leishmaniasis are; sodium stibogluconate, pentamidine or amphotericine B which is toxic and the administration of these drugs may require a prolonged stay in hospital (Chappuis *et al*., 2007). Furthermore, these drugs are expensive and their use is mostly limited in undeveloped and developing countries, Ineffectiveness of drugs against several species of *Leishmania* is another disadvantage (Barbosaa *et al*., 2012). It is not completely understood how these drugs act against the parasite; they may disrupt its energy production or trypanothione metabolism (Azeemi *et al*., 2011).

Life-long immunity to VL has motivated development of prophylactic vaccines against the disease but very few have progressed beyond the experimental stage, No licensed vaccine is available till date against any form of leishmaniasis. High toxicity and increasing resistance to the current chemotherapeutic regimens have further complicated hesitation in VL endemic regions of the world , Advances in vaccinology, including recombinant proteins, novel antigen-delivery systems/ adjuvants, heterologous prime-boost regimens and strategies for intracellular antigen presentation, have contributed to recent advances in vaccine development against VL. Attempts to develop an effective vaccine for use in domestic dogs in areas of canine VL should be pursued for preventing human infection (Das and Ali, 2012).

Previous studies have shown the effect of different types of rays like γ -ray, UV light and laser on *Leishmania* parasite , There are many sites for the application rays for example it's used in vaccination and treatment (Al-Jeboory *et al*., 2007).

Laser is an acronym of light amplification by the stimulated emission of radiation and is advice that convert 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

photocaustic. In several branches of medicine ,laser is used as therapeutic agents such as in ophthalmology , dermatology , gynecology and surgery (Al-obaidi *et al*., 2006)

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. (Cem Sener, 2012).the diode laser emitting laser light at 805 nm , IR light (light in the invisible spectrum below red from 700-2000 nm) and thin flexible glass fiber with a diameter of 8mm , the output power of (0.94, 2.01 and 2.76)W and the power densities were 1.87, 4.0 and 5.49 W/cm^2 for 0.94 , 2.01 and 2.76 W , respectively (Ismail *et al*., 2012).

Laser technology has revolutionized many medical fields , such as 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 .(Kreisler *et al*., 2003) .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 (Jialiang , 2015) .

A helium-neon laser (He-Ne) is a type of gas laser consisting of mixture of helium and neon gas inside the small bore capillary tube. The best known and most widely used He-Ne laser operates at a wavelength of 632.8 nm in the red part of visible spectrum. The He-Ne laser is highly compact, reliable. The mechanism responsible causing bacterial death has been reported to involve the formation of singlet oxygen and free radicals ., He-Ne laser has spatial characteristics such as 632.8 nm of wavelength, good directivity, high intensity, good monochromatic and coherence is a variable low-level laser , The low-level laser has some biology effects such as cell vitality , phagocytosis (Pohekar *et al*.,2013). He-Ne laser was the first widely accessible source of coherent light , has a photobiological nature , has equal efficiency of noncoherent and laser light the biostimulation treatment of stomach and duodenal ulcers (Karu , 1988). Helium :Neon laser of about 9 m W measured power and wavelength 632.8 nm , the horizontal laser beam was reflected vertically on sample pit using a plane mirror (Kandela *et al*., 2004).

Increasing of radioactive contamination is an important problem of ecology , microorganisms are the simplest and basic part of the biosphere , and their physiological state can serve an indicater of condition of the biosphere as whole . microorganisms can be used as sensors for monitoring the environment radiotoxicity (Kudryasheva *et al*.,2010a; Tatiana *et al*., 2008; Kudryasheva *et al*., 2010b)

Beta rays is a 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 in respectively, Gamma rays is a electronmagnetic 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 then 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 (Mohammed and Ahmmed , 1988) .

Gamma irradiation is electromagnetic 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 (Trampus *et al*.,2006) .DNA is the principal cellular target governing loss of viability after exposure to gamma irradiation, DNA damage occurs predominantly by the indirect action of gamma rays, which interact with other atoms or molecules, particularly water, to produce reactive free radicals, Cell death (defined for proliferating cells as loss of reproductive capability) is predominantly induced by double-strand breaks in DNA, separated by not more than a few base pairs, which can generally not be repaired by the cell (Hall and Giaccial ,2006).

The effect of radiation on cells by carries enough energy to remove electrons from molecules in a cell , thereby the free radicals can be production and cause the damage in most other molecules in a cell, such as DNA or RNA , by oxidizing them (Michelle ,2010).

Gamma and Beta 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 (Katia ,2012). 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 (Calabrese,2000).

The aim of this study was to investigate the effectiveness of Semiconductor , He-Ne laser and Beta ,Gamma irradiation on the viability of *Leishmania donovani* parasite promastigotes **.**

Materials and methods

Leishmania donovani promastigotes cultivation

Parasite cultivation was done according to Ismail *et al.,* (2012) with some modifications as follows:

L. donovani 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 supernatant was removed and the precipitate was resuspended using sterile normal saline (physiological saline), the suspension was mixed using vortex to get homogenous suspension , which compared with the McFarland solution ($1.5*10^8$ CFU/ml) , then 1 ml of this solution was exposed to Semiconductor in (5, 10, 20, 30) minute with wavelenghth 532 nm , exposure to He-Ne laser in(5, 10, 20, 30) minute with wavelenghth 6328 A° and exposure to Beta, Gamma irradiation of **^{137} CS** isotopes in dose $1.776*10^{-4}$ **µ**sV of Beta ray (energy 0.514 MeV) and exposure to Gamma ray(energy 0.662 MeV) in dose 96.950 **µ**sV in 2hr. , cesium isotopes (^{137} Cs) that give two type of decay Gamma and Beta Rays , and exposure to ^{90}Sr that give one type of decay is Beta rays (energy 0.198 MeV) in dose 63.100 **µ**sV for time 2hr. 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

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 (Riss *et al.,* 2013). This water insoluble formazan can be solubilized using Dimethyl sulfoxide (DMSO), and the dissolved material is measured spectrophotometrically yielding absorbance as a function of concentration of converted dye (Mosmann, 1983).

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.

MTT assay protocol

L. donovani 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 or 630 nm by microplate 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 (Verma and Dey, 2004).

Results and discussion

This study aims to prove the effect of Semiconductor , He-Ne laser and Beta ,Gamma irradiation directly on the parasite and the possibility of its use in the parasites attenuation to be used for immunization of laboratory animals against infection of *L. donovani* .

After the exposure of *L. donovani* promastigotes to Semiconductor in (5, 10, 20, 30) minute with wavelenghth 532 nm , He-Ne laser in (5, 10, 20, 30) minute with wavelenghth 6328 A° and Beta, Gamma irradiation of ^{137}CS isotopes, in dose $1.776*10^{-4}$ **µ**sV of Beta ray (energy 0.514 MeV) and exposure to Gamma ray(energy 0.662 MeV) in dose 96.950 **µ**sV in 2hr. , cesium isotopes (^{137} Cs) that give two type of decay Gamma and Beta Rays , and exposure to ^{90}Sr that give one type of decay is Beta rays (energy 0.198 MeV) in dose 63.100 **µ**sV . the viability of these cells determined using MTT assay which was shown in table(1) , (2) , (3) . , thus cell viability was decreased with long exposure to Semiconductor , He-Ne laser and Beta ,Gamma irradiation and cell killing was increased with long exposure to Semiconductor , He-Ne laser and Beta ,Gamma irradiation .

Found in Table (2) an increase of energy less response and the reason for that is due the fact that the increase energy means the higher penetration of radiation and interaction is less, than when the energy is less. As well , we find that the percentage of killing by beta higher than the gamma and this is due to the nature of each radiation and by the fact that the beta is acharge partical and its specfic ionizaition higher than the gamma radiation .So we can choose low energy with high activity to get high dose which is efficient to killing *Leishmania donovani* because irradiation effect directly or indirectly on flagellum , DNA , cytoplasmic membrane by absorbance irradiation from this microorganisms and therby cause damage to *Leishmania* .

From table (1, 2, 3) use Semiconductor laser , He-Ne laser and Beta , Gamma irradiation was efficient to killing *L.donovani* , this results help to use this laser and Gamma , Beta irradiation to treatment against *L.donovani* that causes many danger infections them VL that is danger to human.

Table (1): The percentage of viable cell and percentage of killing of *L. donovani* promastigotes after exposure to Semiconductor laser and He- Ne laser .

Absorbance (nm)	Percentage of cell viability and percentage of cell killing exposed to semiconductor laser							
	5 min (killing)	5 min (viable)	10 min (killing)	10 min (viable	20 min (killing	20 min (viable	30 min (killing)	30 min (viable)
490	64.86 %	35.14 %	72.97 %	27.03%	89.18%	10.82%	91.89 %	8.11%
Control.= 0.1 , blank = 0.063 , wavelength = 490 nm								
Absorbance (nm)	Percentage of cell viability and percentage of cell killing exposed to He-Ne laser							
	5 min (killing)	5 min (viable)	10 min (killing)	10 min (viable	20 min (killing	20 min (viable	30 min killing)	30 min (viable)
490	70.27 %	29.73%	86.48 %	13.52 %	89.18%	10.82%	89.18 %	10.82%
Control.= 0.1 , blank = 0.063 , wavelength = 490 nm								

Table (2): The percentage of viable and percentage of killing of *L. donovani* promastigotes after exposure to Beta and Gamma irradiation .

Absorbance 490 nm	Percentage of cell viability and percentage of cell killing exposed to Beta and Gamma irradiation						
	Isotopes	Time of exposure	Type of decay	Dose (µsV)	Energy (MeV)	Percentage of viable	Precentage of killing
L1	^{137} CS	2hr.	ɣ β	$1.776*10^{-4}$ 96.950	0.662 0.514	8.11 %	91.89 %
L2	^{90} Sr	2hr.	β	63.100	0.198	10.82 %	89.18 %
Control.= 0.1 , blank = 0.063 , wavelength = 490 nm							

Also, it was clear that Semiconductor laser , He- Ne laser , and Beta and Gamma irradiation affect on the parasite morphology and motility as shown in figure (1) which revealed the parasites devoid their flagella, which negatively affects their movement , perhaps may affect their ability to penetrate and infect host cells. The flagellar or the promastigote forms are seen in the infected sand fly and in culture media, mosquitoes and bugs but it is only in the sand fly that the parasite reaches the buccal cavity which becomes the insect vector of the parasite. They are motile, slender, organisms measuring 10-15 µm in length with a single anterior flagellum (Stinson *et al.*, 1989).

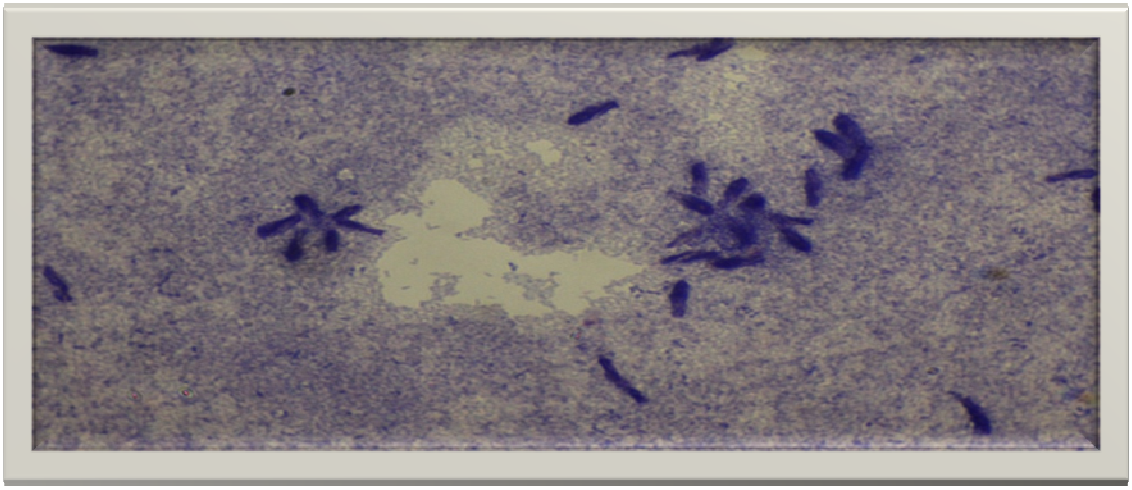

Figure (1): *L. donovani* promastigotes after exposure to laser.

A previous results obtained by Pirnat *et al*.(2011) indicate that the primary mediator of cell death appears to be the interaction between near-infrared spectrum laser light and the bacterial microenvironment, most likely in the form of heating, and suggests that when optimizing the efficacy of laser-assisted endodontic sterilization of the root canal, the optical characteristics of the bacterial microenvironment play a key role, as non-pigmented bacteria appear to be virtually transparent at 808 nm and 1,064 nm.

Al-Obaidi *et al*. (2006) concluded that laser photosensitiser combination had greater efficacy for killing *Leismania* promastigote stage in vitro than laser light alone. They recommended carrying on further studies to use laser photosensitiser combination for treatment of skin lesion of cutaneous leishmaniasis in vivo and a trial of its use for vaccine production against this disease.

Cassagne *et al*. (2014) used matrix-assisted laser desorption ionization time-of-flight mass spectrometry as a promising approach to provide rapid and accurate identification of *Leishmania* from in vitro culture at the species level.

Asilian *et al*. (2004) suggest that cutaneous leishmaniasis can be treated effectively with CO2 laser if those providing the treatment are sufficiently experienced. Laser treatment is more cost-effective than other treatments and can be used as first-line therapy for cutaneous leishmaniasis (wet and dry types). Also AlGhamdi and Khurrum , (2014) proved that fractional CO2 resurfacing represents a safe, effective, and well-tolerated potential treatment for atrophic facial leishmaniasis scars in ethnic skin. And the results obtained by Al-Muslet and Khalid, (2012) showed that the response was excellent in the majority of treated patient (92.3 %),the complications were minimal and transient, the results proved that the low level laser therapy is a successful treatment method for Cutaneous Leishmaniasis and it is easy to perform.

Previous study done by Azeemi *et al*. (2011) who used chromotherapy against Leishmania parasites, whereas red color (644 nm) inhibits the growth and become responsible for the decay of leishmania parasite while orange color (610 nm) increases the growth of parasite. Undoubtedly this makes the procedure of chromotherapy for treatment of leishmaniasis cost effective and easy approachable. The response of *Leishmania* parasite to each color is unique and this confirms Chromotherapy (with 644 nm wavelength), to be very easily manageable by the patient with no problems during the treatment. This kind of study opens new doors for research in bio-sciences and in biotechnology.

Leishmania parasites are thermosensitive , In *invitro* study of AL-obaidi *et al*., (2006) find *L.tropica* multiplied best at 35C° and was completely eliminated at 37C° , therefore both heat and cold treatment have been tried . In Iraq . infrared heat was used to raise the tempreture of Cutaneous Leishmaniasis (CL) lesions to 55C ° for 5 minutes , and all lesions healed in 5 to 6 weeks . Mutinga and Mingola successfully treated three cases of acute CL by combined Ultra violet light and infrared therapy .

The low intensity radiation of He-Ne with wavelenghth 632.8 nm was used successful 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 (trophic ulcers and wounds , inflammations) but very often also "systemic" (Karu , 1988). A privious study of Ismail *et al* , (2012) find He-Ne laser light has an inhibitory action on cariogenic bacteria *Streptococcus mutans* , *Streptococcus sobrinus* , *Lactobacillus casie* and *Actinomyces*

viscosus , photodynamic therapy was used to treat patients with post-surgical infections and abscesses , the bacteria involved being *Peptostreptococcus anaerobes* , *Staphylococcus aureus* and *Streptococci spp.*

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 photosensitization 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 (Husein , 2010 ; Embleton *et al.,* 2002).

Several studies pointed to photodynamic therapy as a treatment alternative for several infections or diseases photodynamic therapy is a technique based on the photosensitization by low-power laser , this technique promots the destruction of the target cell by oxidation mechanisms that lead to cell membrane lysis and protein inactivation (El-Adly *et al.,* 2007). Lately find Kandela *et al*.,(2007) an alternative method of killing pathogenic bacteria is involved in using the red radiation of low power He:Ne laser in presence of sensitizing agent

The lethal effect of ionizing radiation on microorganisms, as measured by the loss by cells of colony-forming ability in semi soild medium, has been the subject of detailed study. Much progress has been made towards identification of the mechanism of inactivation, but there still considerable doubt as to the nature of the critical lesions involved, although it seems certain that lethality is primarily the consequence of genetic damage. 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 (Grezz et al., 1983).

Gamma rays cause damage at a cellular level and are penetrating,VG. However, they are less ionising than Alpha or Beta particles, which are less penetrating. (Rothkamm, 2003). therefore by mechanisms of Gamma and Beta irradiation can be elimination of S.aureus that causes many infection to skin of human , particularly S. aureus infections 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. (Zhu et al., 2008).

A previous study by Lamb et al.,(2002) also Use low dose Gamma irradiation was an effective method for reducing S.aureus , and killing The drugs for the treatment of Leishmaniasis nevertheless , adverse effects when used in first trials in affected populations including other vertebrates such as canniese , being this toxicity mostly related to pancreatic and liver damage (Barbosa et al., 2012).

Conclusion

All laser used Semiconductor laser , He-Ne laser and Beta ,and Gamma irradiation was efficient to killing L. donovani by exposing them to different time and exposing them to different dose and different Sources .

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