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The Effect of He:Ne Laser on Viability and Growth Rate of Leishmania Major

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Abstract: An isolate of *Leishmania major* was grown on the semisolid medium and incubated at 26ºC. The isolate was irradiated by He: Ne laser (632.8 nm, 10 mW) at exposure times (5, 10, 15, 20, 25, 30) minutes in their respective order. The unirradiated groups represent control group. Growth rate and percentage of viability were examined during six days after irradiation. The change in these two parameters reflects the effect of irradiation on the parasite. The results refers that the general growth effected by irradiation in comparison with un irradiation group, The growth rate of parasite decrease with increasing the exposure time in comparison with control group. Parasite viability decrease with irradiation and the percentage of living cell decrease with increasing of exposure time. High doses of laser at long exposure times increase the probability of damage this chromophore and leading to the negative effect on the parasite.

Introduction

Lishmaniasis is an endemic disease in several parts of the world, including south and central America, Africa and Asia (Desjeux, 1992). Protozoan parasites of the genus *Leishmania* are associated with a broad spectrum of diseases, ranging from mild cutaneous to lethal visceral forms (Grimaldi *et al*.; 1993; Shaw *et al*., 1987). The out come of *Leishmania* infection in humans depends largely on the immune responsiveness of the host and the virulence of the infecting parasite strain (Grimaldi *et al*., 1993).

Humans are infected via the bite of sand flies (tiny sand-colored blood feeding flies) that breed in forest areas, caves, or the burrows of small rodents. Wild and domesticated animals and humans themselves can act as a receiver of infection (Desjeux, 1992).

Leishmaniasis are now endemic in 88 countries of the 1.5– 2 million new cases of leishmaniasis estimated to occur annually, only 600,000 are officially declared, nearly 90 % of all cases of cutaneuous leishmaniasis occur in Afghoniston, Brazil, Iran, Peru, Saudia Arabia and Syria (Jacobson, 2003). People who have CL have one or more sores on their skin. The skin sore of CL will heal on their own, but this can take months or even years. Also the sores can leave ugly scars.

Lesion on cosmetically or functionally important sites such as the face or hands, are best given active treatment (Hepburn, 2003). The sore usually treated with Na- stiboglusnale or Meglumine antimonabe or Liposomal Amphotericin. An alternative treatment used in order to get earlier healing with less toxicity and distortion. Irradiation is one of the most alternative processes for the treatment of CL.,

many researches were presented about the effect of different types of rays like γ- ray, UV light and laser on the parasite. Different laser systems each with its own unique properties are now used for a wide range of dermatological conditions (Goldman, 1982).

The absorption of laser light may lead to photochemical interaction, chromophores interaction or both (Markolf, 1996 and Pratesi *et al*., 1980). Depending on the wavelength of the laser light, the structure of the tissue and doses of irradiation (Chopra *et al*., 1992). The present study aims to investigate the effect of visible He:Ne laser on viability and growth rate of *Lishmania major*.

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.

Rivier *et a*l., (1999) show that it's possible to use the live cell of *Leishmania major* which is attenuated by γ -ray to protect the body against infection by the virulent parasite. On the other side the irradiation used as a treatment. Rodriques *et al*., (1990) treat 10 patients infected with Cutaneous Leishmaniasis with different symptoms with CO_2 laser and their lesions heals in a short time and left less dermal distortions (Rodriques *et al*., 1990).

Materials & Methods

A strain of ***Leishmania major*** was obtained from the research medical center of Al-Khadumia hospital. The strain was cultured in semisolid media which contains NaCl (6.91 g), Cacl2.2H2O (0.22 g), NaHCO3 (0.1 g), ACL (0.29 g), D. glucose (0.77 g). Agar (4 g), peptone (1 g), Beef extract (0.3 g), Defibriunted rabbit blood (200 ml), Ggentamycin (concentration 80 mg/ml) (25 ml) dissolved in 1000 ml of distilled water (Ader *et al*., 1992). Twenty one vials contain 5 ml of semisolid media (for each) were prepared for the experiment and divide into 7 sub-groups (3 for each group), these vials inoculated with 5 x 10^5 parasite/ml, inoculated at 26ºc for 1 /day before exposure them to the laser light. The samples were irradiated with He:Ne laser which operates in a continuous wave mode at 632.8 nm wavelength in the visible region of electromagnetic spectrum and 10 mW power for 5, 10, 15, 20, 25 and 30 minutes exposure time and (0) time representing the control group. After the irradiation, samples were incubated at 26 °C., The total growth rate (parasite/ml) was determine during six days after irradiating using hemocytometer chamber according to:

Total count %(parasite/ml) = parasite No. in 64 square x 2.5 x 10^3 x dilution factor

Viability also was determined using Erythrosine-B viable stain according to (Hodgkinson *et al*, 1980). Each group of irradiation was compared with the control group during six days after irradiation. The laser power density *PD* was calculated from

$$PD = \frac{P_a}{A} = \frac{W}{cm^2}$$

where P_a is the laser power in watt and A is the irradiated area.

Results

Figure (1) illustrates the effect of He:Ne laser on the viable count of the parasite at different exposure times. It is clear that the percentage of viability is highly affected (decreased) with increasing the exposure time of irradiation reaches to the least value 53% at the sixth day after irradiation in comparison with control groups 87%. Figure (2) shows the effect of He:Ne laser on growth rate of *Leishmania major***.** It is clearly seen that the growth rate of parasite decreased with increasing exposure time of irradiation. The rapid decline occurs after the fifth and sixth day when the parasite samples were irradiated for 30 minutes ($4x10^8$) in comparison with control groups ($12.2x10^8$).

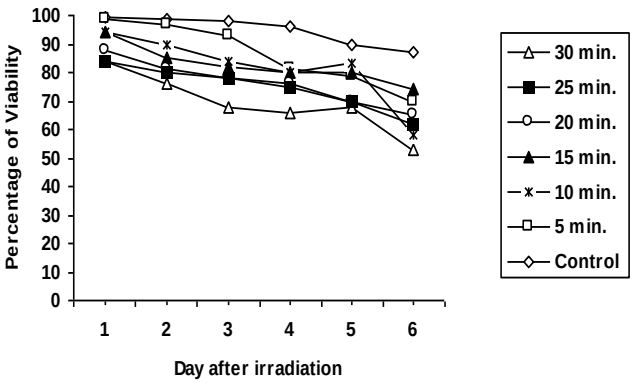

Fig. (1) Effect of He:Ne laser on percentage of viability of *Leishmania major* at PD of 2.04 $x10^{-3}$ W/ cm^2

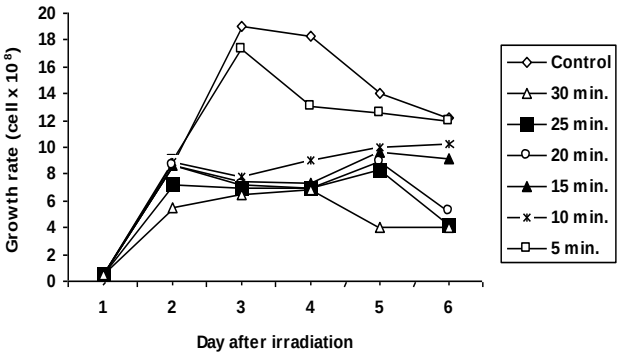

Fig. (2): Effect of He-Ne laser on growth rate of *Leishmania major* at PD of 2.04×10^{-3} W/cm^2

Discussion

In this research changes in the growth rate and viability of the parasite was noticed after laser irradiation.

As it was mentioned by Karu *et al*., (1984) during study the biostimulating action of low intensity monochromatic visible light, the most probable effect of He-Ne laser is may be photochemical interaction due to absorb of He-Ne laser by cytochrom α in mitochondria. Photons enter the tissue and are absorbed in the mitochondria. The photon energy is converted to chemical energy within the cell (Karu *et al*., 1984).

Science the power density is in the range of and exposure time is about (5, 10, 15, 20, 25 and 30) minutes so the probable mechanism is a photochemical (Markolf, 1996).

Adenosine-Tri-Phosphate (ATP) is very important compound for supply the energy for the cell. The production of this compound related to the serial process of oxidation / reduction reactions (redox reactions). Redox reactions are important in cellular process such as cellular respiration (Peter *et al*., 1995). As is clear in figure (1) and (2) the viability and growth rate decreased significantly with increasing of exposure time in comparison with control group, this may be due to effect of high doses of laser that related to the long exposure time (30 minutes) for the certain power density these high doses may affect negatively on the kienetic activities of cytochrome α that is very important in the redox reactions and affect on its ability to take and give the electrons so the production of ATP may be affected leading to the decrease of metabolic activities of the parasitic cell leading to decrease the viability and growth rate. The same results were obtained in the manner of study of the effect of He:Ne laser on the immunological function of phagocytic cell, after higher laser radiation with He:Ne laser phagocytic activity of cells is decreased (Luza *et al*., 1996).

Although of the negative effect of the He:Ne laser, there is a noticeable increase in the growth rate of the parasite at the exposure times 5, 10 minutes for 3 days after irradiation this effect may be explained as a biostimulative action of the laser at low power densities.

The energy of absorbed photons is chemical transfer to redox center of enzymes complexes of the respiratory chain and therefore they more easy oxidated respiratory chain and the ATP synthesis are increased (Sidlova *et al*., 2001).

The rapid decline in the growth rate of the parasite that's significantly appear after 5 day after irradiation at long exposure time is may be due to the accumulation of the metabolic products of the parasite in the medium leading to the toxic effect to the living cells of the parasite.

Conclusions

1. He:Ne laser has a significant effect on growth rate and viability of *Leishmania major*.
2. Reduction viability with laser light may cause increase in growth rate at certain exposure time.

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تأثير الليزر المرئي على حيوية ومعدل النمو للشمانيا

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الخلاصة

تم تنمية عزلة *Leishmania major* على وسط خاص شبه صلب وحضن بدرجة حرارة 26 م° ، ثم جرى تشعيعها بليزر الهليوم : نيون بطول موجي 632,8 نانومتر وقدرة 10 ملي واط ولأزمان تشعيع (5 ، 10 ، 15 ، 20 ، 25 و30) دقيقة بالتعاقب بالمقارنة مع مجموعة السيطرة . درست كل من حيوية ومعدل نمو الطفيلي خلال 6 أيام بعد التشعيع ، أن التغير في زمن التعريض عكس هذا التغير على حيوية ومعدل نمو الطفيلي. النتائج المستحصلة اظهرت ان النمو العام للطفيلي كان له التأثير عند التشعيع بالمقارنة مع مجموعة السيطرة ومعدل النمو للطفيلي قلت بزيادة زمن التشعيع بالمقارنة مع مجموعة السيطرة وحيوية الطفيلي ومعدل الخلايا الحية قلت بزيادة زمن التشعيع. ان تأثير ليزر الهليوم : نيون يمكن ان يكون ناتج عن امتصاص الليزر من قبل متحسسات للضوء (سايتوكرومات في المايتوكوندريا) مسببا تغيرات فيزيائية و كيميائية لجزيئات المستقبلات الضوئية في السلسلة التنفسية ، حيث يؤثر أنزيم السايتوكروم في المايتوكوندريا على النشاط الكيموحياتي و العمليات الايضية داخل الطفيلي الذي يؤدي بدوره الى انخفاض حيوية ومعدل النمو .