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The effect of laser light exposure on survival and viability Of *Leishmania major* amastigote : *in vitro* study

***Hussein Saher AL-Obaidi ,**Abdullah Hussein AL-Jeborey**
* Dept. of Medicine, College of Medicine, Tikrit University, Tikrit, Iraq
**Dept. of Biology, College of Education, Tikrit University, Tikrit, Iraq

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Abstract
This study was designed to evaluate the effectiveness of laser light radiation alone and laser-photosensitiser combination against *Leishmania major* amastigote in vitro. The result shows that laser-photosensitiser combination was more effective in killing than laser light alone. This effect may be photochemical due to absorption of laser light wavelength by exogenous photosensitiser which cause bond breaking leading to toxic effect on the amastigotes.

تاثير التعرض لاشعة الليزر على بقاء وحيوية الطور اللاسوطي لطفيلي اللشمانيا الكبيرة (اللشمانيه ميجور) دراسه خارج الجسم الحي

حسين ساهر العبيدي عبدالله حسين الجبوري

المستخلص
صممت الدراسه لتقدير فاعلية اشعة الليزر لوحدها واشعة الليزر مع المحسس الضوئي ضد الطور اللاسوطي للشمانيا الكبيرة خارج الجسم الحي. اظهرت النتائج ان الليزر مع المحسس الضوئي اكثر فاعليه في القتل من الاشعه لوحدها. هذا التاثير للمعالجة الضوئيه قد يعزى الى امتصاص الطول للموجي لاشعة الليزر بواسطه المحسسات الضوئية الخارجيهالتي تعمل على زيادة فعاليه الاشعه بالنفوذ داخل الطفيلي وتحطيم الاواصر ممايؤدي الى اظهار التاثير السمي على الطفيلي.

Introduction

Laser is routinely used in many branches of clinical medicine, such as in gynecology, dermatology, surgery and in ophthalmology(1). There have been conducted several reports of the ability to kill microorganisms in vitro. Mathews and Sistrone (2) demonstrated that a toluidine blue-sensitised colourless mutant of *sarcina lutea* could be killed by polychromatic light from tungsten and fluoresent lamps. Wilson (3, 4) reported that various toluidine blue O sensitised oral bacteria like *Prophyromonas gingival and Fusobacterium mucleatum* could be killed by low dose of helium-neon laser light.In Iraq, AL-Obaidi (5) demonstrated that providine-iodine sensitised *Staphylococus aureus* isolated from wounds could be killed by light from helium-neon laser. Thus this study conducted to evaluate the killing effect of laser on *L.major* amastigote.

Materials and Methods

Parasite

The parasite, isolated initially from patients with cutaneous leishmaniasis attended to dermatology department at Tikrit Teaching Hospital, then the parasite was typed using enzyme electrophoresis. They were maintained on semi-solid medium and subcultured every 21 days.

Laser

The laser was laser diode gas laser with a measured output of 5mW (Laser Becaon, INC, Michigan. USA) was used in the present study. This emits light with a wavelength of 630 nm in a collimated beam with a diameter 1.3 mm.Povidine iodine was used as a photosensitiser.

Expermental

Attempt was made for the killing of *L. major* amastigote by using laser light alone and in combination with povidine iodine as photosensitiser.

For experimental purposes, amastigote was grown in liquid media at 35C for 6 days. After 6 days of incubation heavy growth was obtained, then the amastigotes were harvested and resuspended in an equal volume of phosphate buffer saline to reach a final concentration of million amastigotes/ml. Aliquots (100µl) of the suspension was transferred to a sterile test tube and an equal volume of povidine iodine solution was added to each tube to give a final concentration equal to 0.002 µg/ml. The test tube was placed on a magnetic stirrer and exposed to laser light for 2, 4 and 6 minutes. Control tubes containing the promastigotes suspension plus saline solution in place of the povidine iodine solution were treated in an identical manner to determine the effect of laser radiation alone on amastigotes viability. A further four tubes, identical to those described above, were prepared and these were not exposed to laser light. Each run was done in duplicate. The vials were then incubated at 35C for 5 days (2, 5).On the next five days the culture were counted. A 1 : 10 dilution in saline together with the appropriate dye was prepared. The dye for amastigotes was 0.4% Trypan blue. The amastigotes permeable to the blue dye are dead while viable ones exclude the dye (6). The chamber of a Neubauers slide is charged and the number of organism in 16 s mall corner square are counted.

The total number per ml= N (counted) x 10 (number in mm)x 1000 (number in 1 ml) x 10 dilution factor).

GI %= No. of treated amastigotes divided by No. of untreated amastigotes (control) multiply by 100.

Results

Exposure to laser alone and laser-photosensitiser combination for 3 and 6 minutes have no effect on the

number and growth index, therefor, its results were neglected. The significant results were obtained after exposure for 9 minutes.

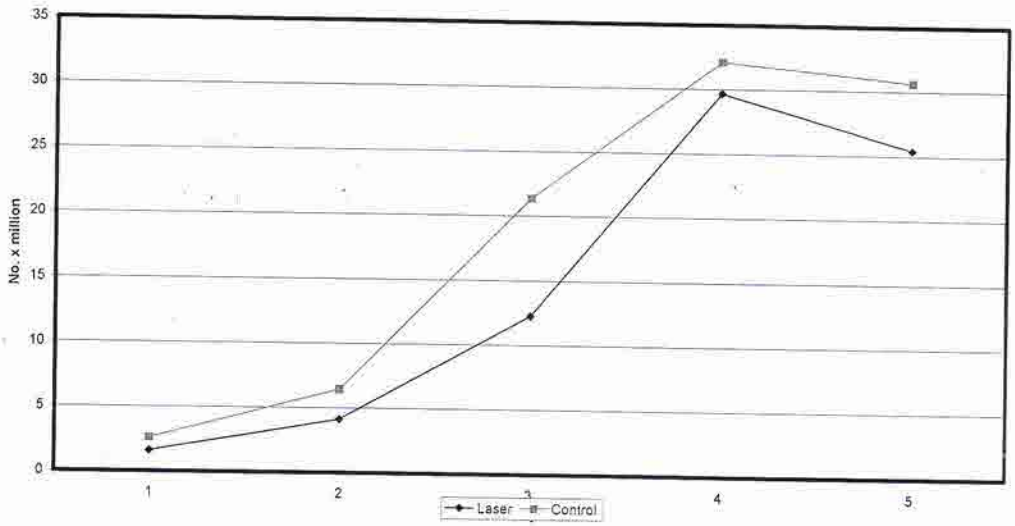

Fig. 1 shows the effect of laser light on the number of *L. tropica a*mastigotes

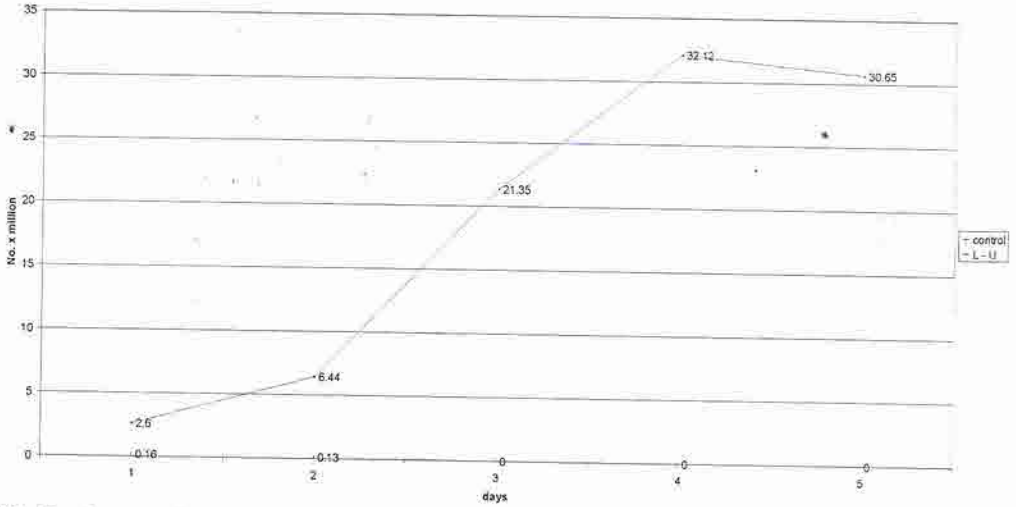

Fig.2 shows the effect of laser-ultrameladinine combination exposure on the number of *L.major*.

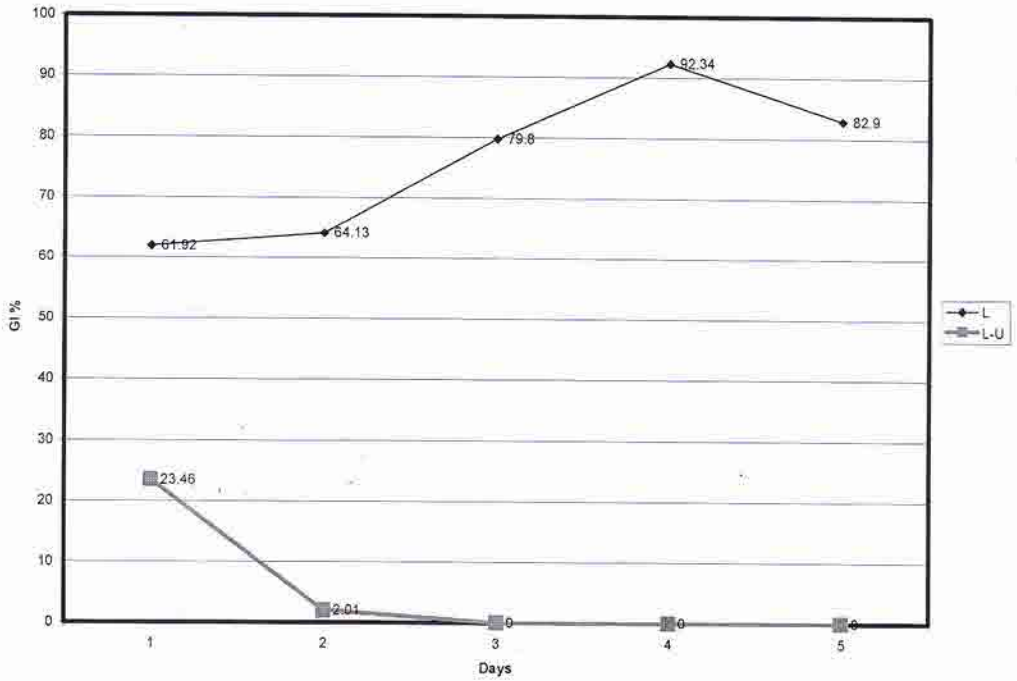

Fig.3 shows the effect of laser and laser-photosensitiser exposure on the growth of *L..major*

Discussion

Results of the present investigation clearly indicate that laser light alone and laser-photosensitiser combination inhibits the growth of amastigotes of *L. major* in vitro.It is obvious that the laser-photosensitiser combination was the most effective in killing when compared with laser light alone. Fig. 1 and 2 shows that the laser-photosensitiser combination was more effective in reducing the number of live amastigotes than laser light alone. It was found that the total number of amastigotes was zero at exposure with laser-photosensitiser while corresponding value for laser alone was12.25million amastigotes/ml.These results can be explained by that, the laser light lead to photochemical change in the cells, these changes can occur as a results of direct excitation and electronic bonds by light. This excitation can lead to rearrangement of molecular bonds and the formation of molecular fragments and finally resulting in cell death. The presence of photosensitiser act to increase the absorption of light by cells and lead to increasing in their effect (7, 8).Thus from this finding it was suggested that laser source may be used as a new approach of treatment for Cutaneous leishmaniasis.However, the above conclusion unable for application unless, in vivo evaluation for laser light effectiveness as therapeutic approach for *L.major* to be performed. At this time we are investigating the effectiveness of laser light exposure as treatment for *L. major* in vivo.

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