

**THE EFFECT OF LASER AND LASER PHOTOSENSITIZER
COMBINATION ON LEISHMANIA TROPICA PROMASTIGOTES IN
VITRO**

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ABSTRACT

Cutaneous leishmaniasis is a common disease in Iraq, especially in the central part of the country. Several treatments have been suggested for this disease but none is completely effective and without side effects. Several research studies are focused on the development of alternative treatments.

The study was carried out in College of Medicine, Tikrit University, to show the effect of laser and laser photosensitiser (ultrameladine) combination on Leishmania tropica promastigotes in vitro, after exposure for 3, 6 and 9 minutes. It was found that exposure to light from 5 mw lasers in the presence of ultrameladine lead to destruction of L. tropica promastigotes in vitro. The effect of laser and laser photosensitiser was greater than laser light alone. Their effect on the parasite after exposure for 9 minutes was significantly greater than exposure for 3 and 6 minutes.

**تأثير أشعة الليزر و ليزر التراميلادينين على اللشمانيا الجلدية البروماستيكوت
(promastigotes) في الزجاج**

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الخلاصة

اللشمانيا الجلدية هي من الأمراض الشائعة في العراق وخاصة في المنطقة الوسطى من البلاد.

أقترحت علاجات كثيرة لهذا المرض ولكن ليس فيهم علاج فعال وبصورة كاملة وبدون أعراض جانبية. كثير من الدراسات البحثية تركزت لإيجاد العلاجات البديلة. أجريت الدراسة في كلية الطب- جامعة تكريت، لبيان تأثير أشعة الليزر وأشعة الليزر مع التراميلادينين على الطور البروماستيكوت (promastigots) للشمانيا الجلدية في الزجاج.

أثبت أن التعرض لأشعة الليزر بوجود محلول التراميلادينين قد أعطى أفضل النتائج في قتل طفيلي اللشمانيا من أشعة الليزر وحده. وجدت أن تأثيرهما عند التعرض لمدة (9) دقائق كانت أكثر من التعرض لمدة 3 و6 دقائق. وجدت أن التعرض من (5mw Laser) ليزر بوجود التراميلادينين يؤدي الى قتل الطفيلي في الزجاج.

INTRODUCTION

Laser is an acronym of light amplification by the stimulated emission of radiation and is advice that convert electrical energy in to light energy (1).

Laser beam have three special characteristics that differentiate them from ordinary light. Monochmaic (all of the same color or wavelength), collimated (emitted in a narrow parallel beam) and coherent (all the light waves are in synchrony). As a result of collimation there is a very little divergence over long distances (2).

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 tissue. There are three basic types of effect on living tissue such as photothermat (3), photochemical (2) and photo-caustic (2). In several branches of medicine, laser is used as therapeutic agents such as in ophthalmology, dermatology, gynecology and surgery (4).

There have been several conducted reports on the ability of laser to kill the microorganism in vitro. Mathews and Sistrom (5) demonstrated that a toulidine blue-sensitized colorless mutant of *Sarcina lutea* could be killed by polychromatic light from tungsten and fluorescent lamps. Wilson (6, 7 & 8) reported that various toludine blue O (TBO) sensitized oral bacteria like *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Actinobacillus actinomycetemomitans* could be killed by low doses of helium-neon laser light. Macmillan et al., (9) found that gram positive *Sarcina lutea* and gram negative *Escherichia coli* and *Pseudomonas aeruginasa* could be killed by helium-neon light in the presence of the dye. Al-Obaidi (10) found that providine-iodine sensitized *Staphylococcus aureus* could be killed by helium-neon laser.

Leishmania parasites are thermosensitive. In in vitro study, *L. tropica* multiplied best at 35 C and was completely eliminated at 37 C. In view of this feature both heat and cold treatment have been tried. In Iraq, infrared heat was used to raise the temperature of CL lesions to 55 C for 5 minutes, and all lesions healed in 5 to 6 weeks (11). Mutinga and Mingola successfully treated three cases of acute CL by combined ultraviolet light and infrared therapy (12).

Although laser has been used for treatment of several skin diseases since 1970, but there are controversies about the effect of laser therapy on the processes of wound healing, connective tissue repair and collagen biosynthesis. Rackcheev, et al., (13), found complete clinical cure in patients suffering from skin leishmaniasis with an argon laser. It has been shown that Helium-Neon low energy laser at wave length 630 nm is effective in wound healing (14). Babejev, et al., (15), used carbon dioxide laser to vaporize the local lesions caused by cutaneous leishmaniasis. They found that the treatment reduces the management of patient at 1.5 times and is followed by satisfactory aesthetic outcomes. Eissa, et al., (16) investigated the mechanism of action and efficacy of CO₂ laser rays and hypertonic sodium chloride (NaCl) with different concentration in treatment of cutaneous leishmaniasis. They concluded that CO₂ laser then 7% NaCl are good modalities for cutaneous leishmaniasis treatment. Asilian et al., (17) found that the CO₂ laser was more effective in treating cutaneous leishmaniasis than glucantime (1.12 times), had fewer side effects and resulted in a shorter healing time. In Isfahan, Iran, Asilian, et al., (18) showed that carbon dioxide laser

radiation is highly effective to vaporize local cutaneous leishmaniasis in 24 patients with lupoid cutaneous leishmaniasis.

The study was conducted to show the effect of laser and laser-photosensitiser combination on *Leishmania tropica* in vitro.

MATERIALS AND METHODS

Laser: The laser was diode gas laser with a measured output of 5 mw (Laser Becaon, INC., Michigan, USA). This emits light with a wavelength of 630 nm in a collimated beam with a diameter 1.3 mm.

Photosensitiser: Ultrameladinine (Memphis Co. for Pharm. And Chemical Ind. A.R.E.) was used as a photosensitiser.

Parasite: *Leishmania tropica* promastigotes was obtained from the *Leishmania* unit at the Research Center/ Nahrin College of Medicine.

Experimental design: Attempt was made for the killing of *L. tropica* promastigotes by using laser light alone and in combination with ultrameladinine as photosensitiser.

Promastigotes was grown in liquid media at 26 °C for six days. After 6 days of incubation heavy growth was obtained. On the day of experiment, promastigotes were harvested and diluted to 1×10^6 promastigotes /ml, then the aliquots (100 ml) of the suspension was transferred to a sterile test tube and an equal volume of ultrameladinine solution was added to each tube to give a final concentration equal to 0.002 mg/ml. The test tube was placed on a magnetic stirrer and exposed to laser light for 3, 6 and 9 minutes. Control tubes containing the promastigotes suspension and saline solution instead of ultrameladinine were treated as before, to determine the effect of laser radiation alone on promastigotes viability. A further four tubes as those described above, were prepared, were not exposed to laser light. Each run was done in duplicate. They were incubated at 26 C for 5 days (7 &10).

On the next five days the cultures were counted a 1:10 dilution in saline together with the appropriate dye was prepared. The dye for promastigotes was 0.4 % trypan blue. The promastigotes permeable to the blue dye are dead while viable ones exclude the dye. The chamber of Neubaus slide is used for counting the promastigotes (19).

The total number per ml= No. counted x 10 (number in mm) x 1000 (number in ml) x 10^9 dilution factor.

Growth index (GI) % = No. of treated promastigotes divided by number of untreated promastigotes multiply by 100.

Statistical analysis: Analysis of variance test was performed to show significant difference between groups.

RESULTS

The number of live *L. tropica* promastigotes after 3, 6 and 9 minutes exposure to laser and laser-photosensitiser is shown in table (1). It is illustrated that the number of promastigotes on day 5 after exposure were 22.75, 16.88 and 24.9×10^6 in laser, laser-photosensitiser combination and control groups respectively.

It was found that on 6 minutes of exposure, the number of promastigotes on day 5 were 22.65, 5.85 and 24.9^6 in laser, laser-photosensitiser combination and control, respectively. On 9 minutes exposure, it was obvious that laser-photosensitiser had more significant effect than laser light alone and the number

of promastigotes on day 5 after exposure were 15.43, 0.85 and 24.9x10⁶ in laser, laser-photosensitiser combination and control groups, respectively.

The growth index of *L. tropica* in vitro, illustrated in table (2). It was found that laser-photosensitiser exposure lead to decrease in growth index from 100% at day 0 to 3% at day five after exposure, while growth index after exposure to laser light alone was 61%. Statistically there was significant difference between them (P<0.01).

Table 1. Effect of laser (L) and laser-ultrameladinin (L-U) on *L. tropica* promastigotes invitro.

Incubation Time of exposure (Minutes)	Light	Day 1	Day 2	Day 3	Day 4	Day 5
3	L	2.7	12.6	24.0	29.6	23.0
	L-U	2.4	8.3	18.2	21.6	17.1
6	L	2.5	12.5	23.6	20.5	22.0
	L-U	2.0	5.2	7.0	8.8	7.0
9	L	2.2	11.6	19.7	19.6	22.2
	L-U	2.0	4.2	3.6	3.0	1.8
Control		2.6	6.44	21.35	32.12	30.65

Table 2. The effect of laser (L) and laser-ultrameladinin (L-U) combination on growth of *L. tropica* promastigotes in vitro on day 5 of exposure.

Time of exposure (minutes)	Laser	Laser-Ultrameladinin
3	91.36	67.79
6	62.57	14.7
9	61.08	3.41

DISCUSSION

Although pentavalent antimony is the treatment of choice for healing the lesions of cutaneous leishmaniasis, but it has a high incidence of side effects. In general, adverse reactions are expected in patients with liver and renal impairment, cardiac arrhythmias and prolonged QT intervals, in small children, in pregnant and breast feeding women and in obese elderly and immunocompromised patients (20). In addition to that lesion of cutaneous leishmaniasis is usually resistant to the conventional therapies for leishmaniasis and it may persist and spread slowly for many years (21). Therefore it is worthwhile to find alternative treatment for destroying the parasite and healing of lesion of cutaneous leishmaniasis.

In literature review, we did not come across the application of laser and photosensitiser combination for treatment of lesion of cutaneous leishmaniasis. In the present preliminary study the effect of laser and laser photosensitiser combination was applied against promastigote stage of the parasite in vitro.

The results of this study have demonstrated that, exposure to light from 5mw laser with ultrameladine (methoxsalen) resulted in significant decline in promastigotes number, and at 9 minutes exposure of their combination lead to complete destruction of promastigotes number. This approach of killing promastigotes is photochemical method (3), which was chosen on the basis of literature report and criteria which used for killing of microorganisms (9 &10).

It is concluded that laser photosensitiser combination had greater efficacy for killing of promastigote stage of parasite than laser light alone. As the results of in vitro study is not applicable for treatment of cutaneous leishmaniasis in vivo. It is recommended to carry 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.

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