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Research article

The effect of Nd: YAG lasers on *Leishmania donovani* promastigotes

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ABSTRACT

The present study was conducted to assess the effect of Nd:YAG laser on *Leishmania donovani* promastigotes. *L. donovani* promastigotes exposed to Nd:YAG laser (wavelength 1060 A°) in 500, 1000 and 1500 pulse between each pulse and another 6 second in comparison to the control (*L. donovani* promastigotes without exposure to Nd:YAG laser. The effect of Nd:YAG laser on the viability of *L. donovani* promastigotes was measured using the colorimetric MTT assay and the percentage of viability was counted. *L. donovani* viable cells were decreased with long exposure to Nd-YAG laser. Nd:YAG laser was effective in killing most of *L. donovani* promastigotes, and the remaining were devoid flagellum that may lost their ability to cause infection.

Keywords: Attenuated promastigotes, *Leishmania donovani,* Nd: YAG laser.

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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 [1]. Among several drugs used in the treatment of leishmaniasis such as sodium stibogluconate, pentamidine or amphotericine B which is toxic and the administration of these drugs may require a prolonged stay in hospital [2]. 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 [3]. is not completely understood how these drugs act against the Parasite; they may disrupt its energy production or trypanothione metabolism [4].

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

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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 [5].

Previous studies had shown the effect of different types of rays like γ -ray, ultraviolet (UV) light and laser on *Leishmania* parasite. There are many sites for the application rays for example it's used in vaccination and treatment [6].

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 [7]. Laser technology has revolutionized many medical fields, Nd:YAG lasers are used in ophthalmology to correct posterior capsular opacification, a condition that may occur after cataract surgery, and for peripheral iridotomy in patients with acute angle-closure glaucoma, where it has superseded surgical iridectomy. Frequency-doubled Nd:YAG lasers (wavelength 532 nm) are used for panretinal photocoagulation in patients with diabetic retinopathy [8]. 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 [9]. They are also used to reduce benign thyroid nodules [10], and to destroy primary and secondary malignant liver lesions [11,12]. This study aimed to investigate the effectiveness of Nd: YAG laser on the viability of *L. donovani* parasite *in vitro*.

MATERIALS AND METHODS

Parasite cultivation & laser exposure

Parasite cultivation was done as follows: *L. donovani* promastigotes were cultivated in M199 media at 25̊ C for five days to reach the stationary-phase culture, then culture was centrifuged (5000 rpm for 10 min). The pellet was suspended in 150 ml of sterile normal saline ,then 1 ml of this solution was exposed to different pulses (500,1000,1500 pulse) of Nd:YAG laser in comparison to control group (without exposure). Each run was done in triplicate, re-diluted 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 [13]. 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 [14].

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. donovani promatigotes was prepared in 96-well plates in a final volume of 100 µl/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 h at 25˚C. The media was removed and 100 µl of DMSO solution was added in order to solubilize the formazan crystals. The plate was stirring gently then, left for 15 min. 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 [15].

Statistical analysis

Chi-square test was used to assignment the difference between two groups. $P < 0.05$ was considered as significant difference and $P < 0.01$ as highly significant difference.

RESULTS

This study aims to prove the effect of Nd-YAG laser directly on the parasite and the possibility of its use in the parasites attenuation to be used for immunization of laboratory animals against visceral leishmaniasis.

After exposure of *L. donovani* promastigotes to different pulses (500, 1000 and 1500 pulse) of Nd:YAG laser, the viability of these cells determined using MTT assay which was shown in **table 1**. Statistical analysis revealed significant (both $P<0.05$ and $P<0.01$) differences between different number of pulses, thus cell viability was decreased with long exposure to Nd-YAG laser, and killing cells were increased with long exposure to Nd:YAG laser .

Also, it was clear that Nd-YAG laser affects on parasite morphology and motility (**Fig. 1**) which revealed the parasites devoid their flagella, which negatively affects their movement and perhaps may affect their ability to penetrate and infect host cells.

DISCUSSION

It has been suggested previously that high-power lasers, such as the Nd:YAG laser, may be useful for destroying

Table 1. The percentage of viable *Leishmania donovani* promastigotes after exposure to Nd-YAG laser.

Absorbance (nm)	Percentage of cell viability and cell killing after exposed to Nd-YAG laser					
	500 pulse (killing)	500 pulse (viable)	1000 pulse (killing)	1000 pulse (viable)	1500 pulse (killing)	1500 pulse (viable)
490	86.0 %	14 %	89.9 %	10 %	97. 6 %	2.4 %
630	91.0 %	9 %	91.5 %	8.5 %	99.7 %	1.3%

χ^2 Tab.= 5.991 (P< 0.05), χ^2 Tab.= 9.21(P<0.01), χ^2 Cal.= 4.233

bacteria, presumably by a thermal effect. The bactericidal action of a high-power Nd:YAG laser on a suspension of *E. coli* was studied. In a thermocouple equipped cuvette, a temperature rise up to 50 ºC was observed after the use of laser with a power output of 100 Watt for 23 second [16].

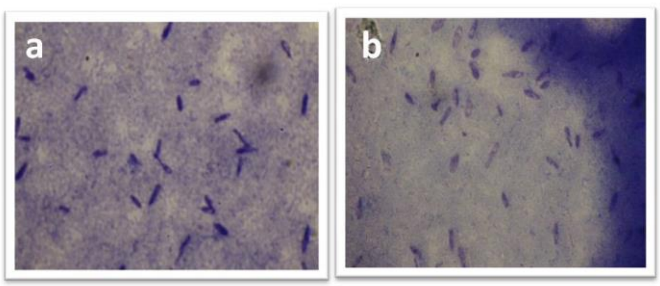

Fig 1. A and B showed *L. donovani* promastigotes after exposing to Nd-YAG laser (stained with giemsa stain).

A previous results obtained by previous study [17] 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.

Nd-YAG laser has been introduced long ago in medicine (1973). It has great energy and penetrates deep into tissues. It is used for ablation of oral leukoplakia with very good subsequent results. More pronounced thermal effect and pain during the procedure, requiring anesthesia, should be considered as negative aspects of the treatment with this type of laser [18, 19] showed that the treatment of oral leukoplakia through the application of Nd-YAG laser is characterized with good therapeutic effect and smooth postoperative period with no significant pain and discomfort, making it an appropriate solution in complex treatment of the disease.

Previous results demonstrate that the 1064-nm long-pulsed Nd:YAG laser could be a safe and effective treatment modality in the management of patients with onychomycosis, subjects receiving multiple treatments achieved better results than those receiving only one treatment. These data suggests that the 1064 nm Nd:YAG Pin Pointe Foot Laser should be considered as an effective alternative therapy to the typical oral and topical medications. Furthermore, the treatment is quick and easy to incorporate into a practice, yielding high patient satisfaction and increased revenues [20].

Also, disinfection of contaminated canals by different laser wavelengths, while performing root canal therapy furthermore various laser wavelengths, particularly of diode and Nd:YAG lasers can be effective in reducing intra canal microbial count. Maximum effect is obtained when laser light is used in canals in combination with sodium hypochlorite irrigating substance in appropriate concentration. Therefore use of laser energy can improve success rate of root canal treatments [21].

Al-Obaidi *et al.* [22] 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.* [23] 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.* [24] suggest that cutaneous leishmaniasis can be treated effectively with CO_2 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, [25] proved that fractional CO_2 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 [26] 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.

In previous study, Azeemi *et al.,* [27] 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. From this study it can be concluded that Nd:YAG laser was effective in killing most *L. donovani* promastigotes, and the remaining of them were devoid flagellum that is why they may be not being able to cause infection.

Conflict of interest

The authors declare that they have no conflict of interests.

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Unicorn