



Evaluation of the efficacy of UV light, Laser lights and *Nigella sativa* alcoholic extract on the Viability of *Leishmania tropica* promastigotes *in vitro*

¹Haneen E. Hussein , ²Abdul Rahman A. Al-Tae , ³Mohammed A. Kadir

^{1,2}College of Medicine / Tikrit University / Tikrit / Iraq

¹haneen_ emad @yahoo.com , ²abdulrahman @ yahoo.com

³College of Medicine / Kirkuk University / Kirkuk / Iraq

³mohammdsalam @ yahoo.com

Received date: 15 / 12 / 2014

Accepted date: 8 / 3 / 2015

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 researches are focused on the development of alternative treatments. The present work aimed to evaluate the efficacy of ultra violet light, laser lights, and Nigella sativa alcoholic extract on the viability of Leishmania tropica promastigote in vitro.

The study included exposure Leishmania tropica promastigote stage in vitro cultured to 2, 4, 6, 8 minutes of ultra violet light and laser lights, also exposure to 40, 50, 60, 70 mg/ml concentrations of Nigella sativa alcoholic extract, then evaluated their effects on replication number and parasitidal rate of parasite.

It was found that there was significant decrease at level($p \leq 0.05$) in replication number of Leishmania tropica promastigotes groups which exposed to 40, 50, 60, 70 mg/ml of alcoholic extract of Nigella sativa, while there was significant decrease at level($p \leq 0.05$) in replication number of groups exposed to 6, 8 minute of laser helium neon light and in groups exposed to 4, 6, 8 minute of laser diode light, and there was no significant decrease at level($p \geq 0.05$) in replication number of groups exposed to 2, 4, 6, 8 minute of ultra violet light in comparison with control groups on the day 5 of incubation. There was increased in parasitidal rate of Leishmania tropica promastigote stage with increasing the time and the concentration of exposure to radiation lights and the alcoholic extract, it was 0%, 25%,

47%, 57% on 2min,40mg/ml respectively and reached to 4%, 68%, 86%, 90% on 8min,70mg/ml respectively under exposure to ultra violet light, laser helium neon light, laser diode light and *Nigella sativa* of alcoholic extract on the day 5 of incubation.

It can be concluded that the efficacy of *Nigella sativa* alcoholic extract was significantly higher than that of ultra violet light, laser helium neon light, laser diode light in decreasing the replication number, and increasing the parasitidal rate of *Leishmania tropica* promastigote stage.

Keywords: Ultra violet light, Laser helium-neon light, Laser diode light, *Nigella sativa* alcoholic extract, Viability of *Leishmania tropica* promastigote.

تقدير كفاءة الأشعة فوق البنفسجية، أشعاعات الليزر، والمستخلص الكحولي للحبة السوداء على حيوية اللشمانيا الجلدية بطور promastigote

حنين عماد حسين¹ ، عبد الرحمن عزيز الطائي² ، محمد عبد العزيز قادر³

^{1,2}كلية الطب / جامعة تكريت / تكريت / العراق

¹haneen-amad@yahoo.com ، ²abdulrahman@yahoo.com

³كلية الطب / جامعة كركوك / كركوك / العراق

³mohammdsalam@yahoo.com

تاريخ قبول البحث: 2015 / 3 / 8

تاريخ استلام البحث: 2014 / 12 / 15

الملخص

اللشمانيا الجلدية من الأمراض الشائعة في العراق وخاصة في المنطقة الوسطى من البلاد. علاجات كثيرة أقرحت لهذا المرض ولكن ليس فيهم علاج فعال وبصورة كاملة وبدون أعراض جانبية. كثير من البحوث تركزت لأيجاد العلاجات البديلة. يهدف العمل الحالي الى بيان كفاءة الأشعة فوق البنفسجية، أشعاعات الليزر و المستخلص الكحولي للحبة السوداء على حيوية اللشمانيا الجلدية بطور البروماستيكوت. الدراسة تضمنت تعريض اللشمانيا الجلدية بطور البروماستيكوت في المزرعة الخارجية الى 2، 4، 6، 8 دقائق من الأشعة فوق البنفسجية وأشعاعات الليزر، كذلك

عرضت الى 30، 40، 50، 60، 70 mg/ml تراكيز المستخلص الكحولي للحبة السوداء، ثم قدرت كفاءتهم على عدد التكاثر ومستوى المقتول من الطفيلي.

وجد نقصان معنوي عند المستوى ($P \leq 0.05$) في عدد تكاثر مجاميع اللشمانيا الجلدية بطور البروماستيكوت التي عرضت الى 40، 50، 60، 70 mg/ml من المستخلص الكحولي للحبة السوداء، بينما يوجد نقصان معنوي عند المستوى ($P \leq 0.05$) في عدد تكاثر المجاميع المعرضة الى 6، 8 دقائق لأشعة ليزر هيليوم نيون وفي المجاميع المعرضة الى 4، 6، 8 دقائق من أشعة ليزر دايبود ولا يوجد نقصان معنوي عند ($p \geq 0.05$) في عدد تكاثر المجاميع المعرضة الى 2، 4، 6، 8 دقائق من الأشعة الفوق البنفسجية بالمقارنة مع مجاميع السيطرة في اليوم 5 من الحضان. يوجد زيادة في مستوى المقتول من اللشمانيا الجلدية بطور البروماستيكوت مع زيادة وقت وتركيز التعرض الى اشاعات الأشعة والمستخلص الكحولي، انها 0%، 25%، 47%، 57% تحت 2min,40mg/ml بالتتابع وتصل الى 4%، 68%، 86%، 90% تحت 8min,70mg/ml بالتتابع تحت التعرض الى الأشعة الفوق البنفسجية، اشعاع ليزر هيليوم نيون، ليزر دايبود والمستخلص الكحولي للحبة السوداء في اليوم 5 من الحضان.

نختم بالقول أن كفاءة المستخلص الكحولي للحبة السوداء أحصائيا أعلى من الأشعة الفوق البنفسجية، أشعة ليزر هيليوم نيون، أشعة ليزر دايبود في تقليل عدد النمو وزيادة نسبة القتل للشمانيا الجلدية بطور البروماستيكوت.

الكلمات الدالة: الأشعة الفوق البنفسجية ، أشعة ليزر هيليوم نيون ، أشعة ليزر دايبود ، المستخلص الكحولي للحبة السوداء ، حيوية اللشمانيا الجلدية بطور البروماستيكوت.

1.INTRODUCTION

Cutaneous leishmaniasis is a common disease in Iraq, caused by obligate intracellular, kinetoplastid protozoa of the genus *Leishmania* (Trypanosomatidae) [1].

The parasite exists in biphasic forms, the flagellated promastigote found in vector and culture media and the non-flagellated amastigote which live in macrophage of mammalian host and axenic culture media [2]. Many alternative treatment modalities have been proposed and screening of medicinal plants for antileishmanial activities is a very effective way to find new active substances [3]. Effects of the UV light on progression of disease have been documented, before 1935; broad- spectrum UV light sources were shown to affect small pox,

lupus vulgaris, and erysipelas [4]. Because such sources emit UV–A and UV–C as well as UV–B, their effects, cannot be attributed to UV-B only, more recent studies on herpes and leishmaniasis used lamps emitting UV-B only, Mutinga and Mingola successfully treated three cases of acute cutaneous leishmaniasis (CL) by combined ultraviolet light and infrared therapy [5]. Laser can be classified on the basis of the power output into two types; high power laser which includes: CO₂ laser, organ laser and low power laser which includes: helium neon laser, gallium arsenide diode laser and semiconductor diode laser [6].

There have been several conducted reports on the ability of laser to kill the microorganism *in vitro*. Al-Obaidi found that providing-iodine sensitized *Staphylococcus aureus* could be killed by helium-neon laser [7], it has been shown that helium-neon low energy laser at wave length 630 nm was effective in wound healing [8]. On the other hand in the medicine, laser was used as therapeutic agents in ophthalmology, dermatology, gynecology and surgery [9]. The chemical and pharmacological studies in the last fifteen years proved the therapeutic activities of many plants, Iraq is considered one of the countries in which a lot of plants, of medical use, grow widely like: *Citrullus colicyn*, *Thymus vulgaris*, *Glycerrhi aglobra*, *Nigella sativa* [10].

Nigella sativa is an annual dicotyledonous of Ranunculaceae family known commonly as "Black cumin" in English, it is a herbaceous plant that grows in Iran and other middle east countries and has been widely used as condiment in pickles, bread and other foods, it has been used as anti-inflammatory, anti-parasitic disease, anti-allergic, anti-cancer and also used as circulatory and immune system support [11].

This study was conducted to show the effect of ultra violet light, laser lights, versus to alcoholic extract of *Nigella sativa* on viability of *Leishmania tropica* promastigote *in vitro*.

2.MATERIALS AND METHODS

An experimental study was carried out in Salah Aldeen government from 13th of November / 2013 to 13th of February / 2014.

UV: Ultra violet light was type B obtained from the light source output 325 nm on the distance 3 cm between it and culture.

Laser lights: Two types of laser lights were used, both of them were directly joined with electrical source to obtain continuous light, the first one was laser helium neon with a measured output of 1 mw and a wave length of 633 nm, and the second one was laser diode

with a measured output of 5 mw and a wave length of 650 nm on the distance 3 cm between the laser lights and culture.

Plant extraction procedure: The alcoholic extract of *Nigella sativa* seeds was prepared according to Grand route [12], and concentrations 40, 50, 60, 70 mg/ml of alcoholic extract were prepared and used in this study.

Parasite: The parasite isolated from patients attended to dermatology department of Tikrit Teaching Hospital whom they complained from skin lesion mostly in exposed part of the body as the face, leg, arm, and clinically diagnosed as cutaneous leishmaniasis.

Culture media: Cultures were carried out using Roswell park medium institute (RPMI 1640) with 10% fetal calf serum (FCS), 100 U/ml of penicillin, 100 mg/ml of streptomycin and 25 U/ml of Nystatin and the pH was justified to 7.2 [2].

Experimental design: Randomized trial was made for evaluation of the effect of UV, Laser lights on the viability of *L. tropica* promastigotes in comparison with alcoholic extract of *Nigella sativa*.

Promastigote were cultured in RPMI 1640 medium with 10% fetal calf serum, antibiotic solution, pH (7.2) and incubated at 27 °C for six days, after 6 days of incubation heavy growth was obtained. Promastigotes were harvested by centrifugation at 3000 rpm for 15 minutes, then mixed with 2 ml of distilled water forming turbid solution. Aspirated 0.3 ml from turbid solution (suspension) and inoculated it into six sterile test tubes containing 6 ml RPMI 1640 medium. Number of promastigotes were used to seed *in vitro* cultures was 3×10^6 cells per 0.3 ml, so each tube has the same chance for promastigotes growth for experimental study.

These tubes were incubated at 27 °C for six days, then examined by using 40 X power of compound microscope, the number of promastigotes were counted with heamocytometer (Neubauer counting chamber (WBC chamber)) with 10 X objective of light microscopy [13]. To determine the effect of ultra violet light, laser lights and alcoholic extract of *Nigella sativa* on the viability of promastigotes, used five sterile tubes for each type of lights, one considered as control and others exposed to lights for 2, 4, 6, 8 minutes, also used five sterile tubes one treated as control and others exposed to 40, 50, 60, 70 mg/ml concentrations of alcoholic extract of *Nigella sativa*.

All cultures were incubated at 27 °C for five days; the numbers of parasites were counted daily under 40 X power by using WBC chamber, the viability of parasite counted by using the following tests [1]

-Replication number = total number of promastigotes in 64 small square of haemocytometer / 0.625×10^6

-Parasitocidal rate = number of unviable promastigote / total number (viable and un viable promastigote) x 100.

The promastigotes permeable to the blue dye are unviable, while viable ones exclude the dye, 0.4% trypan blue was used for detection the viability of promastigotes [2]

3.RESULTS

Table (1) shows the replication number of promastigote stage was highly affected (decreased) with increasing the exposed time and concentrations of *Nigella sativa* alcoholic extract in comparison with control groups on the day 5 of incubation. There was significant decreasing ($P \leq 0.05$) in replication number of groups exposed to laser diode for 4, 6, 8 min and groups exposed to 40, 50, 60, 70 mg/ml of extract, while there was significant decreasing ($p \leq 0.05$) in replication number of groups exposed to laser helium neon only on 6, 8 min and there was no significant decreasing ($p \geq 0.05$) in replication number of all groups exposed to 2, 4, 6, 8 min of ultra violet light.

Table (1): The effect of ultra violet, laser helium-neon, laser diode light and *Nigella sativa* alcoholic extract on the replication number of *L. tropica* promastigotes *in vitro* on the day 5 of incubation.

Exposure time,con.of <i>Nigella sativa</i> alcoholic extract	Ultra violet light	Laser helium neon light	Laser diode light	<i>Nigella sativa</i> alcoholic extract
control	128.8 ± 58.6	106.9 ± 19.1	98.9 ± 21.8	188.4 ± 52.2
2min, 40mg/ml	129.1 ± 58.6	89.0 ± 19.8	71.4 ± 22.3	122.4 ± 54.1*
4min, 50mg/ml	128.3 ± 57	83.7 ± 20.2	66.1 ± 21.4*	102.4 ± 54.7*
6min, 60mg/ml	127.9 ± 57.9	74.9 ± 19.3*	61.6 ± 20.6*	97.0 ± 52.6*
8min, 70mg/ml	129.2 ± 57.6	70.4 ± 19,5*	55.7 ± 19.9*	75.9 ± 48*

-The reading represent the Mean values \pm Standard deviation for replication number for six experiments.

- * There was significant effect at level ($p < 0.05$).

Table (2) shows that the effect of ultra violet light, laser helium neon light, laser diode light was lower than the effect of *Nigella sativa* alcoholic extract on increasing the parasitidal rate with increasing the exposed time and concentration of extract. On the day 5 of exposed to 2 minute of ultra violet light, laser helium neon light, laser diode light and 40 mg/ml of *Nigella sativa* alcoholic extract was 0%, 25%, 47%, 57% respectively and reached to 4%, 68%, 86%, 90% respectively in groups which exposed to 8 minute of ultra violet light, laser helium neon light, laser diode light and 70 mg/ml of *Nigella sativa* alcoholic extract.

Table (2): The effect of ultra violet light, laser helium neon light, laser diode light, and *Nigella sativa* alcoholic extract on the parasitidal rate of promastigote stage on the day 5 of incubation

Exposure time, con. of <i>Nigella sativa</i> alcoholic extract	Ultra violet light	Laser helium neon light	Laser diode light	<i>Nigella sativa</i> alcoholic extract
2min , 40mg/ml	0 %	25 %	47 %	57 %
4min, 50mg/ml	0 %	37 %	57 %	67 %
6min, 60mg/ml	2 %	57 %	66 %	77 %
8min, 70mg/ml	4 %	68 %	86 %	90 %

-The reading represents the parasitidal rate of six experiments.

4.DISCUSSION

These results revealed that there was no significant decreasing in replication number at level ($p \geq 0.05$) for promastigote stage exposed for 2, 4, 6, 8 min to ultra violet light in comparison with control group and there was significant decreasing in replication number at level ($p \leq 0.05$) on promastigotes groups exposed for 6, 8 min to laser helium neon in comparison with control group, laser diode cause significant decreasing in replication number at level ($p \leq 0.05$) on promastigote groups exposed for 4, 6, 8 min in comparison with control

group, while alcoholic extract of *N. sativa* cause significant decreasing in replication number at ($p \leq 0.05$) on all exposed concentrations as in Table (1).

Parasiticidal rate of extract increased to 90% on promastigote group exposed to 70 mg/ml of extract, while reached to 4, 68, 86% respectively on promastigote groups exposed to 8 m of ultra violet light, laser helium neon light, laser diode light, as demonstrated in Table (2).

These findings revealed that the decreasing in the replication number of *Leishmania tropica* parasite which caused by alcoholic extract of *Nigella sativa* was significantly higher than that caused by radiation lights in spite of the major constituents of *Nigella sativa* seed (nigellin, metarbin, melanthin, glycosides, saponines, volatile oils, fixed oil, albuminous proteins, glucose, mucilage resins) which provide the source of nutrient for parasite when cultured [14], this due to the antileishmanial effect of the extract which attributed to compounds belong to diverse chemical groups such as benzyloquinolines, B-carboline alkaloids, steroidal glycosides and quinines, which caused inhibition of protein synthesis or DNA synthesis leading the decrease of metabolic activities of the parasitic cell and leading to decrease the viability [15], These results agreed with the study of Jarallah *et al* (2003), who indicated that aqueous and alcoholic extracts of *Nigella sativa* seeds have antileishmanial effects against *L. major* promastigote *in vitro* [16], agreed with the study of Erzaiqe *et al* (2013), who proved the efficacy of mixture honey and *Nigella sativa* extract in increasing the clinical cure, the residual scar size, and required dose of pentostam in treatment of the lesions caused by *leishmania* [17], agreed with the results of Yahya *et al* (2011), who showed that *Nigella sativa* alcoholic extract has a significant difference on *Escheriechia coli*, *Giardia lamblia*, and have highly inhibitory effect on isolated *Candida albicans* [18].

The effect of radiation lights in decreasing the viability of promastigote of *Leishmania tropica* can be explained by that, the effect was on the kinetic activities of cytochrome α that is very important in the redox reactions and effect on parasite ability to take and give the electrons so the production of ATP may be affected leading the decrease of metabolic activities of the parasitic cell leading to decrease the viability [19], this study was in agreement with Al- Obaidy *et al* (2006), who proved that laser diode light alone and laser photosensitiser combination inhibits the growth of promastigotes of *Leishmania tropica in vitro* [20], and in agreement with Al- Jeboory *et al* (2007), who found that laser helium neon has a significant effect on the replication number and the viability of *Leishmania majore* [21], Abdul- Sada *et al* (2008) found that there was a significant decrease in the hatchability,

survival of eggs and larva of sheep nematode after exposure to ultra violet radiation and this depend on the level of radiation and the periods of exposure [22].

It is concluded that alcoholic extract of *Nigella sativa* had greater efficacy in decreasing the viability of promastigote stage of parasite than radiation lights.

The developing microbial resistance to the existing anti- microbial agents has become a serious problem. Therefore, production of new potent agent is urgently needed, especially for hospitals and health centers. Furthermore, studies on the anti- microbial potential of plant extracts, active ingredients and/ or chemical drugs should be tested on experimental animals.

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AUTHOR



Haneen E. Hussein: graduate of the Technical College / Kirkuk/ Dept. of Medical Laboratories on of the year (2004), and employee in the Dept. of Health in (2005) and currently now a master student in Tikrit University/ college of medicine since (20012).