



In vitro Investigation the Antifungal and 940 nm Diode Laser Effects on Inhibition of Candida Albicans Isolated from Oral Cavity

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Abstract

Background: Candida albicans is a prevalent commensal that can cause severe health problems in humans. One such condition that frequently returns after treatment is oral candidiasis. **Aim:** the goal of this research is to evaluate the efficiency of 940 nm as a fungicidal on the growth of Candida albicans in vitro. **Material and Methods:** In vitro samples (fungal swabs) were taken from the oral cavity of 75 patients suffering from oral thrush. Following the process of isolating and identifying Albicans. The samples are divided into four groups:(Group 1): Suspension of C. albicans was put in a solution of saline as a control group. (Group 2): Suspension of C. albicans that had been treated with nystatin. (Group 3): Suspension of C. albicans was irradiated by diode laser 940 nm at 1 W for 300 seconds in continuous mode. (Group 4): C. albicans suspension was irradiated by laser in a combination of nystatin. ANOVA, Dunnett t, and LSD tests were used to examine the data. A highly significant statistical variation in the count of C. Albicans before and after therapy. **Results:** The result of this study, finds that the reduction in the CFU/ml in group 4 (laser+nystatin) is highly significant and diode laser has a fungicidal effect on the growth of candida albicans. **Conclusion:** using a 940 nm diode laser (continuous mode) in a combination of antifungal (nystatin) acts as a fungicidal effect on Candida albicans.

Keywords: Candida Albicans, diode laser, 940 nm, Nystatin, oral thrush.

1. Introduction

Oral candidiasis is caused by a fungal infection called Candida albicans, which accounts for 60-70% of all cases (Hussain and Al-Drobie, 2022). Non-pathogenic Oral candidiasis could be caused by the yeast Candida albicans, which was a common member of the oral microbiota. (Al-Ali DA and Al Groosh D,2022). In persons with decreased cellular immunity, C. albicans could become active and cause oral infections (Williams et al,2012). Fungi have been discovered in dental root canals, dentin walls, and even periodontal pockets (Al-Maliky MA et al, 2022).



People who wore poorly fitting dentures were at risk for developing denture stomatitis, a chronic inflammatory condition of the palatal mucosa. (Karkosh ZS et al , 2018; Mawlood ZS and Najj GA,2020; Mohammed HA and Fatalla AA,2020). Even more concerning was the fact that 81% of AIDS patients receiving oral candidiasis treatment had *Candida* species that were resistant to antifungal drugs like fluconazole. (Johnson EM et al,1995). Between one-third and two-thirds of those who wore full dentures suffer from this problem. (Jainkittivong A et al,2010). Treating fungal infections, particularly systemic ones, is notoriously challenging due to therapeutic limitations and the emergence of drug-resistant strains. These findings highlight the importance of continuing to investigate and develop innovative therapies for fungal illnesses. (Chabrier et al,2008; Cowen LE et al,2009; Coleman JJ et al, 2010). Denture stomatitis is difficult to treat due to the multifaceted complexity of the disease's source. Traditional treatments include things like better dental care, antiseptic mouthwash, denture removal and soaking in disinfectant solution at night, and the replacement of ill-fitting dentures. The antifungal drug nystatin is effective in treating denture stomatitis. (Cueto et al,2013).

Nystatin is a Polyenes family member, and it functions by binding to ergosterol (a part of the fungal cell wall). Furthermore, it forms a complex with cholesterol in the cytoplasmic membrane of the host cell. Nystatin has an association with a number of unpleasant side effects, including diarrhea, stomach discomfort, tachycardia, bronchospasm, face swelling, muscular stiffness, Itching, burning, and rashes; Stevens-Johnson syndrome (Hammond SI.,1977). Using nystatin for an extended period of time not only requires the patient's cooperation but also raises the possibility that the fungus *Candida albicans* will develop resistance to the drug (Janeth et al.,2019). Furthermore, the drug is quite expensive (Zomorodian K et al,2011; Orlandini et al,2020). Low-level laser (LLL) is a type of laser that has non-thermal effects on biological systems (Lin et al,2010) and is used in biological systems to promote tissue regeneration and minimize inflammation (Huang et al,2011). The optical window for low-level laser treatment is between 600 and 1100 nanometers (nm), resulting in deeper tissue penetration and a larger cell-light response (Raghavendra,2005). The 940 nm diode laser is often used in dental offices because it is small, easy to get, cheap, and can be used for many different things in oral medicine. This source's radiation has an effect on the biofilm and destroys it (Mustafa and Salah,2020).

The aim of this research was to assess the efficacy of a 940 nm laser with a 1 W power output and a 300 s duration in inhibition of *C. Albicans* colonies.

2. Materials and methods

2.1 Samples collection and preparation

The University of Baghdad's Basic Science and Microbiology Department provided a *Candida albicans* strain isolated from the oral cavity of a patient suffering from oral thrush in order to test the antifungal activity. This strain was grown in a 48-hour incubation at 37°C by plating it on Sabouraud Dextrose Agar. The suspension was diluted in an optical density 0.5 McFarland standard solution. The amount of *C. albicans* that was prepared was 10⁴ viable cells/ml. The suspension (10⁴ cell/ml) was transferred to a 96-well microplate (0.1 ml in each well) using a sampler. Every antifungal activity test was carried out inside the laminar flow hood, at room temperature, in complete darkness, and under sterile circumstances.

2.2 Groups

Four groups were investigated on different microtiter plates:

Group 1: *C. albicans* suspended in saline solution served as a control.

Group 2: a suspension of *C. albicans* that has been treated with (0.1ml) of nystatin solution (100.000 Units/ml).

Group 3: *C. albicans*, irradiated by (940 nm diode laser) only.



Group 4: *C. albicans* irradiated by (940 nm diode laser) in a combination of (0.1ml) nystatin.

2.3 Laser Irradiation

The laser source in use is a 1 W continuous mode diode laser with a wavelength of 940 nm. Irradiation was performed at a 90-degree incidence angle with the laser beam's constantly pointing in a direction perpendicular to the wells' entrance, as in Fig.1



Fig.1: Irradiation procedure.

2.4 Antifungal activity test

A 0.1 ml of a diluted fungal culture containing 1×10^4 CFU/ml was administered to group 2 along with 0.1 ml of nystatin. Following that, (0.1 ml) was seeded on Sabouraud dextrose agar. The dishes had been incubated for 48 hours at 37°C. The number of total colonies of *Candida albicans* (colony forming units CFU/mL) was used to analyze the plates. Using the Swanson, Petran, and Hanlin approach, a sample of colonies between 30 and 300 colonies was chosen for counting. (Swanson et al,2001).

3. Statistical analysis

In order to compare the means of different groups, the gathered data were put through an ANOVA is a one-way analysis of variance. The data was summarised using the mean and standard deviation (SD) and statistical significance was assessed by comparing means and testing for differences using a p-value threshold of 0.05. Multiple Comparisons by Dunnett t-tests were used in order to compare between tested groups and control. SPSS was used for all the statistical analyses (v 20).

4. Results

After isolation and identification of *C.albicans*, radiated by laser as adjacent or assistant to nystatin for biomodulation process, the result was sent to statical analysis. Table 1 demonstrated significant differences between tested means. Table 1 demonstrated significant differences between tested means. The letters A, B, C denoted the degrees of significance, with the most significant beginning with (A) G4(Laser+nystatin)and decreasing with the (C) last (laser alone) .To quantify the significant differences between tested means, the LSD test was performed . Table 2 shows the difference in the statical analysis between groups, compare the colony-forming unit of *Candida albicans* in G1(control) with other groups, it's the highest then laser alone G3 and the lowest one is the G4 (laser + nystatin). The test used in Table 2 is the Dunnett Test which compares the means of two sets of data to determine whether or not there is statistical significance between them.

Table 1. Differences between several groups.

940 nm Laser 1 watt 5 minutes	CONTROL	AF	LASER	L+AF	P value
N	5	5	5	5	0.01 SIG
Mean × 10⁴ CFU/mL	372.60	B 69.20	C 90.80	A 34.40	
Median	372.00	70.00	91.00	35.00	
Std. Error of Mean	1.08	1.02	0.86	1.21	
Std. Deviation	2.41	2.28	1.92	2.70	
Minimum	370.00	66.00	88.00	30.00	
Maximum	376.00	72.00	93.00	37.00	
AF=antifungal L+AF=Laser + antifungal					

Table 2. Descriptive data comparing the CFU/ml of *Candida albicans* in different tested means.

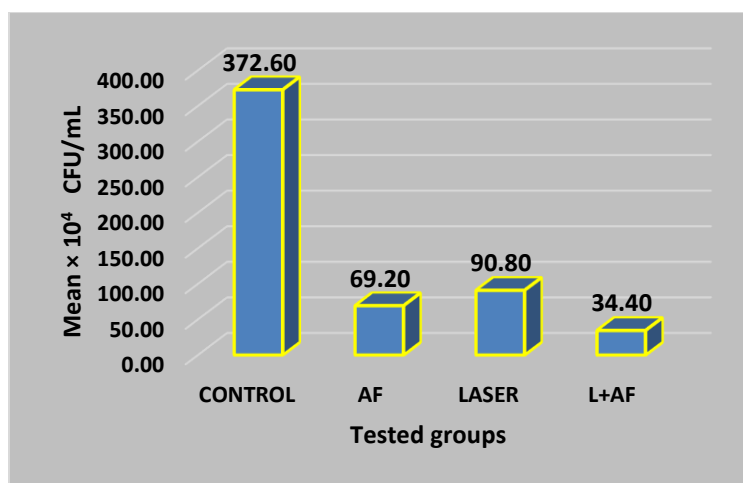
Multiple Comparisons/ 940 nm Laser 1 watt 5 minute						
Dependent Variable: CFU Dunnett t (2-sided)						
GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
AF	CONTROL	303.40	1.45052	0.000	307.2454	299.5546
LASER	CONTROL	281.80	1.45052	0.000	285.6454	277.9546
L+AF	CONTROL	338.20	1.45052	0.000	342.0454	334.3546
AF=antifungal L=Laser L+AF=Laser +antifungal * The mean difference is significant at the 0.05 level.						



Table 3. Time's effect on the CFU/mL.

940 nm Laser 1 watt	CONTROL	100 SEC	150 SEC	200 SEC	250 SEC	300 SEC	P VALUE
N	5	5	5	5	5	5	
Mean × 10 ⁴		E	D	C	B	A	
CFU/mL	372.60	323.40	295.60	254.60	187.80	90.80	
Median	372.00	324.00	295.00	255.00	188.00	91.00	0.001
Std. Error of Mean	1.08	1.08	0.68	0.51	0.86	0.86	SIG
Std. Deviation	2.41	2.41	1.52	1.14	1.92	1.92	
Minimum	370.00	320.00	294.00	253.00	185.00	88.00	
Maximum	376.00	326.00	298.00	256.00	190.00	93.00	

Table 3 exhibited a statistically significant difference between different groups by studying the effect of time on the CFU, showed the significant difference in (300 s) and the lowest one in (100 s). Fig 1. Showed the percentage of mean values of CFU of *Candida albicans*, in G1(control) is a very highest percentage, then the Group of lasers alone(G3), then the percentage of (G2) Group of nystatin alone and the lowest percentage is the Group of (laser+nystatin) G4.

**Fig.1:** Average of colony-forming unit percentage between tested groups.

5. Discussion

Although light's effects have been studied for quite some time, its application as an antifungal agent is more recent. (Mt L et al,2004; Gutknecht N et al ,2000). Many pathogenic microbes have become resistant to conventional antimicrobials, prompting researchers to investigate this phenomenon. The process of acquiring new knowledge by any number of methods, some of which may be therapeutic in nature, is often referred to as "therapeutic." (Tardivo JP et. al,2005; Donnelly RF et. al,2008; Gonzales FP and Maisch T,2012; Oliveira et. al,2014; Bota C and Căruntu B,2015; Meimandi M et al 2017).

The term "therapeutic" refers to the process of obtaining new knowledge through a variety of approaches, including therapeutic techniques. The size of the microbe, the presence of the nucleus, and the

cell wall all contribute to the distinct properties of a fungal cell, emphasising the need to select the appropriate wavelength, power, and time. Group 4 (laser with nystatin) had considerably higher mean values of percentage of CFU compared to the other groups (Table 1). Table 3 exhibited statistically significant changes in CFU with increasing exposure duration, this might be related to the laser's direct action on the cytoplasmic membrane. According to other studies, this one demonstrated that diode laser treatment decreased candida albicans CFU with time. (Mahdi RA and Mohammed AA,2010; Anwer AG.,2005).

In our pilot study, A general mechanism can be developed to explain this phenomenon, one that can account for promoting cell culture activities with visible and infrared lasers at low laser doses, as well as the destructive action of these same lasers at large dosages. In the mitochondria, laser irradiation may result in the creation of a transmembrane electrochemical proton gradient. This increases ATP generation, which activates the Ca^{2+} pump, which depletes the Ca^{2+} concentration gradients of the surrounding media compared to the cytoplasm. This stimulus increases Ca^{2+} entry into cells through the Ca^{2+} ion channels in the plasma membrane. More Ca^{2+} is released from mitochondria via an antiport mechanism when enough irradiation is applied, as a result of the proton motive force (pmf) caused by the proton gradient. Together with other variables regulated by pmf, the increased cytoplasmic calcium induces mitosis and boosts cell proliferation. (Anwer AG.,2005).

Too much Ca^{2+} is released at high laser dosages. Ca^{2+} -ATPase becomes hyperactive as a result, depleting the cell's ATP supply. Certain wavelengths of light can also activate some of the intrinsic components of cells. This allows for the modification of both individual biochemical events and the metabolic activity of entire cells. It is thought that low-power laser effects depend on this kind of reaction. The results of this investigation agreed well with those of previous studies. Mitochondria are vulnerable to exposure to monochrome visible and near-infrared light, as shown by a number of different lines of evidence. (Anwer AG.,2005).

6. Conclusion:

A 940 nm diode laser (continuous mode, 1 W, 300 s) was found to be effective in decreasing the quantity of *Candida albicans* in an in vitro investigation, especially when used in conjunction with nystatin.

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دراسة تأثير ليزر الدايدود (940 نانومتر) و المضادات في تثبيط فطريات المبيضات البيض المعزولة من تجويف الفم في المختبر

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الخلاصة

الخلفية: المبيضات البيض هي نوع سائد يمكن أن يسبب مشاكل صحية خطيرة لدى البشر. أحد هذه الحالات التي تظهر بشكل متكرر بعد العلاج هو داء المبيضات الفموي. الهدف من هذا البحث هو تقييم فعالية ليزر 940 نانومتر كمبيد فعال على نمو المبيضات البيض في المختبر. **المواد والطرق:** تم أخذ عينات (مسحات) من تجويف الفم لـ 75 مريضاً يعانون من مرض القلاع الفموي. بعد عملية العزل والتعرف على فطريات المبيضات البيض. العينات تقسم إلى اربع مجموعات: (المجموعة 1): تعليق المبيضات البيض بوضعها بمحلول ملحي كمجموعة تحكم. (المجموعة 2): تعليق المبيضات البيض وعلاجها بمحلول النيستاتين. (المجموعة 3): تم تشيع معلق المبيضات البيض بواسطة ليزر الدايدود 940 نانومتر عند 1 وات لمدة 300 ثانية في الوضع المستمر. (المجموعة 4): تم تشيع معلق المبيضات البيض بواسطة الليزر مع محلول النيستاتين. تم استخدام اختبارات الاحصاء لفحص البيانات. لوحظ فروقات ذات دلالة إحصائية عالية في عدد المبيضات قبل وبعد العلاج. **النتائج:** بعد التحليل الإحصائي وجد انه انخفاض ملحوظ في عدد المبيضات البيض في المجموعه 4 مع مضاد للفطريات (محلول النيستاتين) وايضا ان ليزر الدايدود يعمل كمطهر فعال ومثبط للمبيضات البيض. **الخلاصة:** استخدام ليزر الدايدود 940 نانومتر (الوضع المستمر) مع مزيج من مضاد الفطريات (nystatin) بمثابة مبيد فعال على المبيضات البيض.

