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Study the Power He-Ne Laser Effect on Dermatophyte *Candida albicans*

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Abstract: Candida albicans is an opportunistic yeast, which is part of the human flora and can cause superficial and systemic infections. He–Ne laser (632.8 nm) in low-level laser therapy was found to modulate the bacteria growth without inducing thermal damage. The effects of He–Ne laser irradiation on the growth and biomass of C. albicans from skin infections were examined in this work**.** Seventy-five skin swabs of 15–40 years old patients were cultured on Sabouraud Dextrose Agar. One strain of C. albicans was irradiated by a He–Ne CW laser at 632.8 nm with exposure times of 10, 30 and 50 s. Colony size, dry weight, daily growth rate, recovery efficiency and morphological modifications were determined. Each treatment was replicated three times**.** Short-term irradiation (10 seconds) significantly inhibited yeast growth, reducing colony diameter to 2.10 ± 0.10 cm, dry weight to 60 ± 2 mg, daily growth rate to 0.53 cm/day, and recovery ratio to 65.6%, compared with the control (3.20 ± 0.12 cm, 85 ± 3 mg, 0.80 cm/day, 100%). Longer irradiation times (30–50 seconds) resulted in partial recovery of growth and biomass, indicating a biphasic response. Morphological examination showed reduced hyphal formation at 10 seconds, whereas colonies at 30–50 seconds resembled the control. The inhibitory effect of He–Ne laser on C. albicans is time dependent, and the most significant suppression occurred after minimum exposure to laser. These findings indicate that low power He-Ne laser could be used as a supplementary antifungal form.

Keywords: C. albicans, He–Ne laser, Low-Level Laser Therapy, Dry Weight

Introduction

Lasers with wavelengths of 550-1000 nm, in the red and infra-red part of the spectrum, are referred to as low energy lasers - or low level lasers. Such lasers are characterized by their penetration of tissue, without incurring thermal damage or structural occupation to organic molecules or cellular components. Its nick name is "cold lasers" since it could interact with the sensitive biological objects without causing a mechanical and thermal damage [1-2]. The dominant interaction of cells with Helium-Neon (He-Ne) laser irradiation is thought to be photochemical rather than thermal. This is the process whereby particular particles are absorbed by cells and this absorption results in biochemical and physiological activity within the cell. Ray of light in the wavelength range of red color are proven to stimulate cellular metabolism; boost cell proliferation as well as fabrics after injury or inflammation [3-4]. Because of these advantages, the He-Ne lasers have been extensively used in different medical fields such as Gynecology, Urology, General Surgery and Dermatology [5] applications till also being applied in Cardiology, Ophthalmology and photocoagulation procedures [6]. Antimicrobial activity of low level laser therapy has become in focus over the last years. Some authors state that the action of laser irradiation may interfere with the microbial cells, by changing membrane permeability, producing reactive oxygen species and interfering with metabolic pathways, what can result in changes on growth patterns and pathogenicity. The mechanisms, however, are not completely understood so far especially in the fungal organisms [5,7]. Candida albicans, which may be detected in the oral cavity region and gastrointestinal system (in addition to vaginal fluid sample), is an opportunistic yeast species commonly found as a member of the normal human microbiota. Though mostly harmless in a healthy person, under immune-compromised conditions or when normal flora become imbalanced it can cause infections. Candidiasis may be superficial, such as those on mucosal surfaces or systemic life-threatening infections [7,8]. Epidemiologic studies suggest that 75% of women will have at least one episode of vaginal candidiasis over their lifetime [8]. Although most Candida infections are amenable to treatment with common antifungal agents, systemic candidiasis continues to be associated with high mortality rates, especially among immunosuppressed and critically ill patients [9-10]. Additionally, the rise in antifungal resistance has posed an increasing clinical problem, indicating the urgency of finding new alternatives or adjunctive therapies. This versa tile pathogenicity of C. albicans is due to various virulence factors such as adherence on medical devices (catheters), formation of biofilm which prevents

the penetration of antifungal agents and secretion hydrolytic enzymes that facilitate invasion into tissues [11-12]. Clinically, patients with candidiasis can experience burning sensation, pain, an altered taste and difficulty in chewing and swallowing when it affects the oral cavity; nevertheless some infections are asymptomatic [13-14]. Because the available antifungal regimes have their inherent limitations and laser has gained much attention as a tool for biomedical ends, elucidating the effects of He-Ne laser irradiation on fungal pathogens could be not only scientifically interested but also clinically relevant. Little is known about the direct impact of He-Ne laser irradiation on growth, virulence, and biofilm formation in Candida albicans and a standardized set of laser parameters for antifungal purposes has not been defined. The current study is, therefore, designed to evaluate the effect of He-Ne laser irradiation on Candida albicans (CA).

Methods

Yeast Collection

Seventy-five skin swabs were taken between 15 - 40 - year old patients who had been clinically diagnosed as having candidiasis and referred to the Azadi Teaching Hospital. The specimens were collected between April and October 2020. All swabs were transferred to the microbiology laboratory aseptically for culture set up.

Candida albicans Separation and Identification

All skin swabs were streaked onto Sabouraud Dextrose Agar (SDA) – a selective media for fungi and incubated at 37 °C for 48 h. Following incubation, fungal colonies were initially isolated according to their physical features such as color, diameter, colony shape and organization of hyphae. Gram staining was also applied, and the smears were viewed under a light microscope to verify Candida species.

Laser Irradiation (He-Ne Treatment)

Pre-irradiation preparation

One isolate which is a confirmed Candida albicans was picked and streaked in Sabouraud Dextrose (SD) broth after incubated from SDA plates. The culture was incubated at 37 °C for four days prior to laser illumination.

laser source were used:

Helium-Neon (He-Ne) laser at 632.8 nm and 1 mW out put power in continuous wave were used.

Irradiation Procedure

Irradiation on solid medium

For the assessment of different laser irradiation times (10, 30 and 50 s), C. albicans grew on SDA was exposed to Laser indication. Irradiation was conducted once daily for four days.

Post-Irradiation Analysis

Measurement of Colony Diameter

On fresh sterile SDA, a 0.5 cm diameter agar disc with laser treated fungal growth was transferred after laser exposure. Control was represented by a similar disc of non-irradiated culture. The plates were incubated at 37 °C for four days and the experiment had three replicates per treatments.

The diameter of the fungal colonies was appraised, and inhibition % was calculated according to:

$$Inhibition\ Ratio\ (\%) = (MDCC - MDTC\ /\ MDCC) \times 100$$

MDCC: Mean diameter of control Colonies.

MDTC: Mean diameter of treated colonies.

Dry weight Determination

Sterile 100 ml SD broth were inoculated with the freshly grown culture (1 % V/V) in 150 ml glass flasks. Yeast inoculation A composite yeast suspension of 4 discs of irradiated yeasts (10, 30 or 50 seconds exposures) per flask with three replicates and unfractioned control inoculums was done. Cultures were grown at 37 °C for four days. After incubation, fungal mycelia were filtered through pre-weighted sterile filter paper. The filter papers were then dried in an oven at 80 °C for 24 h, and the dry weight of fungal biomass was weighed.

Statistical Analysis

Statistical analysis Chi-square (χ^2) test was used for data analysis. Statistical analysis was conducted by Minitab version 17 for results with statistical significance ($p < 0.05$).

Results

Colony Diameter

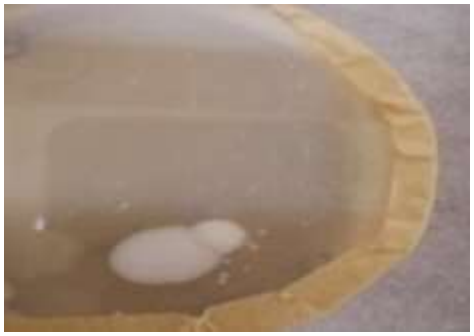
The largest mean colony diameter was recorded in the control group (3.20 cm). After 10 seconds of laser irradiation, the colony diameter decreased to 2.10 cm, representing the smallest growth among all treatments. When the irradiation time increased to 30 seconds, the mean diameter increased to 2.85 cm. At 50 seconds of exposure, the diameter further increased to 3.05 cm, approaching the control value. These results show that short laser exposure (10 sec) reduced fungal growth, whereas longer exposure (30 and 50 sec) allowed greater colony development, Table 1 and Figures 1,2.

Table 1. Mean colony diameter of *C. albicans* after He–Ne laser irradiation

Treatment	Irradiation time	Mean colony diameter (cm) ± SD
Control	0 sec	3.20 ± 0.12
T1	10 sec	2.10 ± 0.10
T2	30 sec	2.85 ± 0.15
T3	50 sec	3.05 ± 0.14

(a): colony growth of control group.

(b): colony growth of 10 S irradiation.



(c): colony growth of 30 S irradiation.

(d): colony growth of 50 S irradiation.

Figure 1. Colony growth at different exposure times

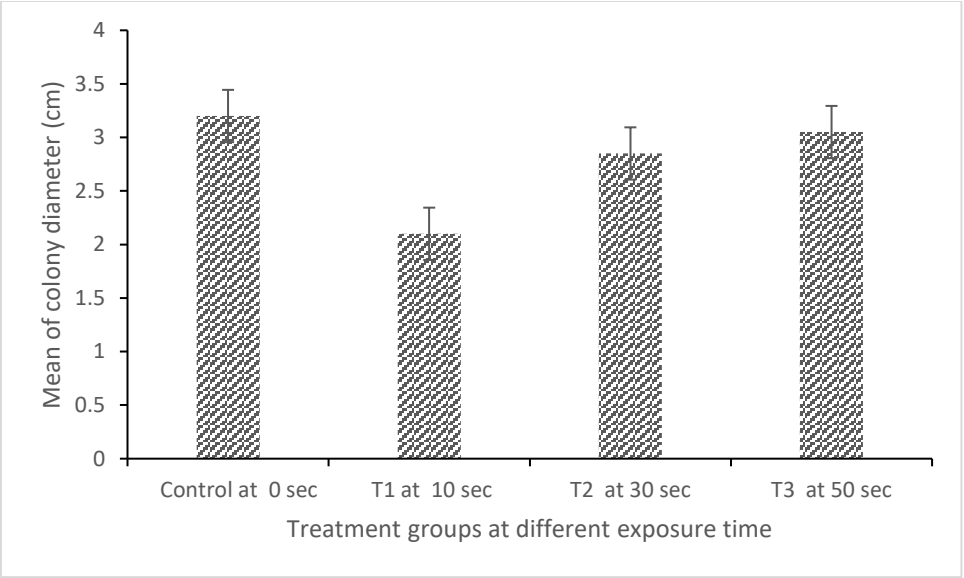


Figure 2. Mean colony diameter of *C. albicans* after He–Ne laser irradiation

Inhibition Percentage of Colony Growth

The highest inhibition of fungal growth (34.4%) was observed at 10 seconds of laser irradiation. When the exposure time increased to 30 seconds, the inhibition dropped markedly to 10.9%. At 50 seconds, the inhibition further decreased to only 4.7%. This confirms that the strongest suppressive effect of the laser occurred at the shortest exposure time, Table 2, Figure3.

Table 2. Growth inhibition percentage of *C. albicans*

Treatment	Irradiation time	Inhibition (%)
T1	10 sec	34.4 %
T2	30 sec	10.9 %
T3	50 sec	4.7 %

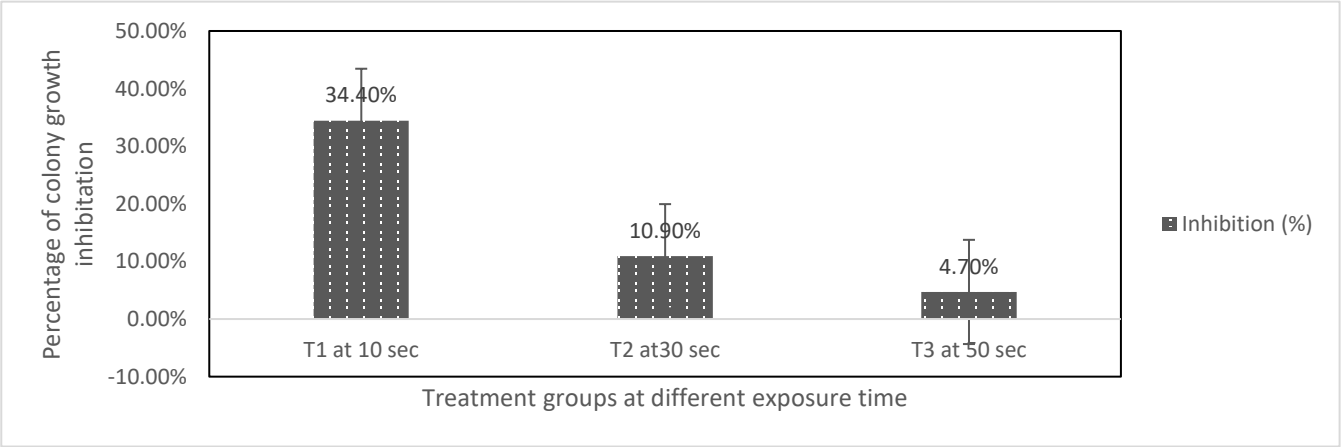


Figure 3. Inhibition Percentage of Colony Growth.

Dry Weight of Fungal Biomass

The control group showed the highest fungal dry weight (85 mg), indicating maximum biomass production. After 10 seconds of irradiation, dry weight decreased significantly to 60 mg, representing the lowest biomass among all treatments. At 30 seconds, dry weight increased to 78 mg, and at 50

seconds it rose further to 82 mg, approaching the control value. This pattern matches the colony diameter results, showing reduced fungal growth at 10 seconds and recovery of growth at longer irradiation times, Table 3, Figure 4.

Table 3. Dry weight of *C. albicans* after laser exposure

Treatment	Irradiation time	Mean dry weight (mg) ± SD
Control	0 sec	85 ± 3
T1	10 sec	60 ± 2
T2	30 sec	78 ± 3
T3	50 sec	82 ± 3

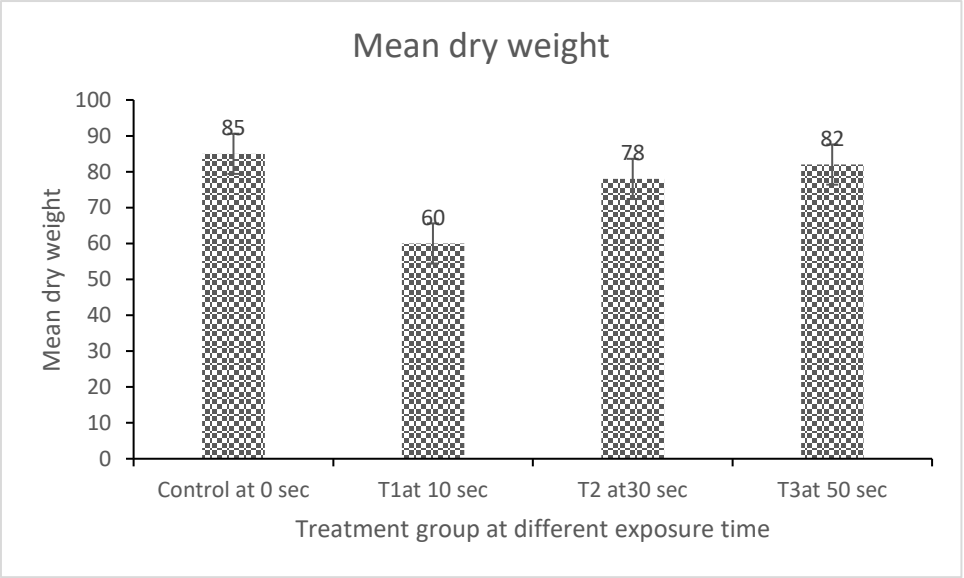


Figure 4. Dry weight of *C. albicans* after laser exposure

Effect of He–Ne Laser Irradiation on Candida albicans Growth, Biomass, and Morphology

The effect of He–Ne laser irradiation on Candida albicans was evaluated through colony diameter, inhibition percentage, dry weight, daily growth rate, and recovery percentage. The results demonstrated a clear time-dependent response. The control colonies, which were not exposed to laser, reached a mean diameter of 3.20 cm with a dry weight of 85 mg, serving as the reference for growth. Short-term irradiation of 10 seconds produced the strongest inhibitory effect, with the smallest colony diameter of 2.10 cm, the lowest dry weight of 60 mg, the highest inhibition percentage of 34.4%, a reduced daily growth rate of 0.53 cm/day, and a recovery ratio of only 65.6% compared with control. This indicates that brief exposure effectively suppressed fungal growth and biomass accumulation. When the irradiation duration was increased to 30 seconds, the colony diameter increased to 2.85 cm, dry weight to 78 mg, inhibition dropped to 10.9%, daily growth rate rose to 0.71 cm/day, and recovery ratio reached 89.1%, showing partial restoration of growth. Further exposure of 50 seconds resulted in a colony diameter of 3.05 cm, dry weight of 82 mg, inhibition of 4.7%, daily growth rate of 0.76 cm/day, and recovery ratio of 95.3%, nearly reaching control values. Morphologically, colonies irradiated for 10 seconds were smaller, less dense, and exhibited reduced hyphal formation, whereas colonies irradiated for 30 and 50 seconds appeared similar to the control, indicating partial to full recovery of hyphal development. The low standard deviations across all replicates confirm the reproducibility of the results, and statistical analysis showed that the inhibition observed at 10 seconds was significant ($p < 0.05$). Overall, these findings indicate that He–Ne laser irradiation has a biphasic effect on C. albicans, with the most effective growth suppression occurring at short exposure, while longer irradiation promotes partial recovery of growth and morphology, Table 4, Figure5.

Table 4. Effect of He–Ne laser irradiation on colony diameter, inhibition, dry weight, daily growth rate, and recovery percentage of C. albicans

Treatment	Irradiation time (sec)	Colony Diameter (cm ± SD)	Inhibition (%)	Dry Weight (mg ± SD)	Daily Growth Rate (cm/day)	Recovery (%)
Control	0	3.20 ± 0.12	–	85 ± 3	0.80	100
T1	10	2.10 ± 0.10	34.4	60 ± 2	0.53	65.6
T2	30	2.85 ± 0.15	10.9	78 ± 3	0.71	89.1
T3	50	3.05 ± 0.14	4.7	82 ± 3	0.76	95.3

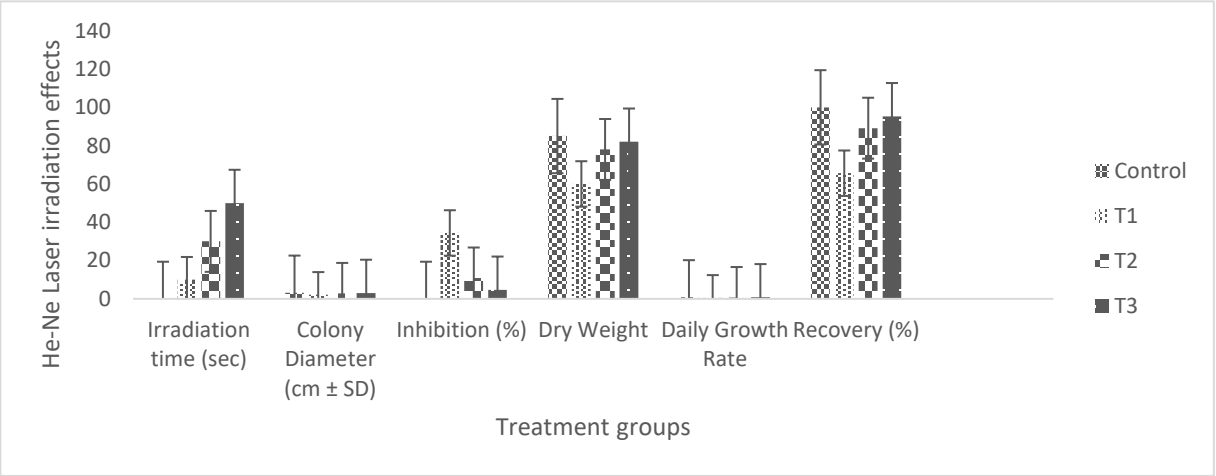


Figure 5. Effect of He–Ne laser irradiation on colony diameter, inhibition, dry weight, daily growth rate, and recovery percentage of C. albicans

Discussion

The findings of present investigation reveal that He–Ne laser irradiation modulates growth rate (colony diameter, dry weight, daily growth rate, recovery %) of Candida albicans in a time-dependent manner. Inhibition of growth was most prominent with brief exposure (10 s) and there was partial recovery of the growth with longer exposures (30–50s), indicating a biphasic nature in the response of yeast cells to laser irradiation. Our results corroborate the previous data demonstrating that growth of Candida albicans could be modulated by low power light. Wilson and Mia [15] also showed that C. albicans becomes more killable on exposure to low-power light under controlled irradiation. In the same vein, Bown and Lovell indicated on how Photodynamic therapy (PDT) that involves light in combination with photosensitizers can lower fungus viability by causing reactive oxygen species generation [16]. Our study did not involve the photosensitizers but as light dependent events could affect microbial growth, [28] which corresponds with our observation that colony diameter and dry weight of UV treated strain was significantly reduced at low exposure durations. In addition, Al-Khazragi and Al-Samaraee indicated that laser light is able to trigger apoptosis in lymphocytes, suggesting thus that low-level laser radiation has an effect on basic cell processes [17]. Although we did not measure apoptosis per se in yeast, the repression of growth rates observed at 10 seconds might reflect perturbations to cell replication or metabolism. The potential photobiological processes were supported by the finding of Vladimirov and coworkers who found that therapeutic laser effects at low-radiation doses are essentially photochemical rather than thermal with concomitant modulation of enzyme activity, cell signaling, and mitochondrial function [18]. Karu (1995) pointed out that low-power laser irradiation can produce electron excitation in photoacceptor molecules, e.g., cytochrome c oxidase and affect mitochondrial metabolism and intracellular signaling [19]. These mechanisms may be responsible for the fact that the longer exposures (30–50 seconds) induced a partial recovery of growth and biomass, since yeast cells have appeared to become accustomed to low-radiation doses. Fedoseyeva et al. reported that exposure

to He–Ne laser has altered yeasts' reproduction and protein synthesis, consistent with our results of decreased colony diameter, dry weight during shorter irradiation time[20]. Moreover, Ferreira et al. showed that He–Ne laser irradiation had a fungal growth inhibitory effect in experimental paracoccidioidomycosis lesions, thereby offering an in vivo evidence for the antifungal activity of low-level lasers [21]. Our data, although obtained in vitro on C. albicans are in line with these observations, and clearly indicate that the accurate modulation of exposure time is crucial to ensure effective growth inhibition. the present results suggest that He–Ne laser irradiation may modulate C. albicans growth and morphology in a time-related manner, being more effective after short exposures. Several lines of evidence indicate that light emitted by a low power laser interacts with cellular components and can modulate growth, metabolism and viability [15–21]. The underlying molecular mechanisms such as reactive oxygen species, expressions of genes or biofilm inhibition still need to be investigated in order to optimize these laser protocols for future clinical treatment of fungal infection.

Conclusions

Time-dependence inhibitory effect of He–Ne laser irradiation on candida albicans: the most effective exposure time of He–Ne laser is 10 seconds as assessed by colony diameter, dry weight and hyphal length. However longer exposure periods (30–50 s) resulted in partial growth recovery, thus displaying a biphasic response. These findings also indicate the possible use of low level He–Ne laser as an adjunct antifungal treatment.

Limitations

Our experiment was an in vitro study focusing on a single yeast isolate and generalisability to other strains might be limited. The molecular level of the laser effect and biofilm form responses were not investigated. Further, the potency of combination therapy with antifungal drugs or photosensitizer was not tested which may be directly translate to effectiveness in the clinical setup.

References

[1] Abdullah SH, Mokhtari-Dizaji M, Hormozi-Moghaddam Z, Bakhshandeh M, Nilforoshzadeh MA. Laser Photobiomodulation of Human Dermal Fibroblasts Combined With Plasma Rich in Growth Factors and Platelet-Rich Plasma: A Comparative In Vitro Analysis. Photodermatol Photoimmunol Photomed.2025;41(6):e70063.
[2] Abdullah SH, Mokhtari-Dizaji M, Hormozi-Moghaddam Z, Bakhshandeh M, Nilforoshzadeh MA. Effects of Low-Level Gallium-Aluminum-Arsenide Laser Therapy on Human Dermal Fibroblast Proliferation, ATP, and ROS Levels. J Lasers Med Sci. 2025 26;16:e27.
[3] Ball K. Lasers: The perioperative challenge. 2nd ed. Nabcy L. Coon, USA; 1995.
[4] Nambiar KR. Laser Principles, Types and Applications. New Age International P Limited Publishers; 2004.
[5] Molero G, Diez-Orejas R, Navarro-Garcia F, Monteoliva L, Pla J, Gil C, Sánchez-Pérez M, Nombela C. Candida albicans: Genetics, dimorphism and pathogenicity. Int Microbiol. 1998;1:95–106.
[6] Ferrer J. Vaginal candidosis: Epidemiological and etiological factors. Int J Gynecol Obstet. 2000;71:21–7.
[7] Naris B. World of Microbiology and Immunology. Detroit, MI: Gale Publishing; 2003. Vol. 1.
[8] Ciurea CN, Irina-Bianca K, Anca DM, Felicia T, Ionela A, Adrian M. Candida and candidiasis—opportunism versus pathogenicity: a review of the virulence traits. J Microorganisms. 2020;8:857–74.
[9] Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev. 2011;36:288–305.
[10] Sardi JC, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes MJS. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol. 2013;62:10–24.
[11] Hoshi N, Mori H, Taguchi H, Taniguchi M, Aoki H, Sawada T, et al. Management of oral candidiasis in denture wearers. J Prosthodont Res. 2011;55:48–52.
[12] Bakhshi M, Taheri JB, Shabestari SB, Tanik A, Pahlevan R. Comparison of therapeutic effect of aqueous extract of garlic and nystatin mouthwash in denture stomatitis. Gerodontology. 2012;29:680–4.
[13] Wilson M, Mia N. Sensitization of Candida albicans to killing by low power light. J Oral Pathol Med.

1993;22(8):354–7.
[14] Bown SG, Lovell LB. The biology of photodynamic therapy in the gastrointestinal tract. Gastrointest Endosc Clin N Am. 2000;10(3):533–50.
[15] Al-Khazragi R, Al-Samaraee I. Effect of laser light on lymphocyte apoptosis. Iraqi J Med Sci. 2008;6(1):45–51.
[16] Vladimirov YA, Osipov AN, Klebanov GI. Photobiological principles of therapeutic applications of laser radiation. Biochemistry (Mosc). 2004;69(1):81–90.
[17] Karu TI. Low power laser therapy. In: Tuan Vo-Dinh T, editor. Biomedical Photonic Handbook. Boca Raton, FL: CRC Press; 2003. p. 48-1–48-25.
[18] Fedoseyeva GE, Karu TI, Letokhov VS, Lobko VV, Pomoshikova NA, Lyopunova TS, Meissd MN. Effect of He-Ne laser irradiation on the reproduction and protein synthesis in yeast. Laser Chemistry. 1984;5:27–33.
[19] Ferreira MC, Brito VN, Gameiro J, Costa MR, Vasconcellos EC, Cruz-Hofling MA, Verinaud L. Effects of HeNe laser irradiation on experimental paracoccidioidomycotic lesions. J Photochem Photobiol B. 2006;84(2):141–9.