Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 3 Vol 8 No 1 2023

Article

Study the effect of the different doses from the laser on *Staphylococcus aureus* Bacteria growth in vitro

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Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.03.74

Abstract

Background: Laser is a novel physical therapy technique used to treat various conditions, including wound healing, inhibition of bacterial growth, and postoperative wounds. High-power pulsed alexandrite laser therapy is one of the most prevalent forms of laser therapy, which is a noninvasive method for treating various pathological conditions, thereby enhancing functional capacities and quality of life. It is a modern medical and physiotherapeutic technology. Generally, the Alexandrite laser emits infrared light with a wavelength of 755 nm, allowing it to propagate and penetrate tissues. *Objective:* This study focused on the application of a high-power pulsed alexandrite laser in vitro to evaluate the effect of a pulsed alexandrite laser on antibiotic-resistant bacteria utilizing varying exposure times, pulse durations, and laser fluencies to determine which dose is more effective on S. aureus bacteria. *Method*: The laser used in this study was the alexandrite laser which was considered a pulsed laser and had the following parameters: The wavelength was 755 nm, the beam diameter was (14 mm), the exposure times varied (30, 60, 90) seconds, the laser fluency (5, 10, 15 and 20 J.Cm-2). The study was carried out after the bacteria were diagnosed as being antibiotic-resistant. They were exposed to different doses of Alexandrite laser. Three samples of bacteria were exposed to laser beams for 30 seconds with a 5ms pulse duration and with a laser fluency of 5J/cm2, and this process was repeated with laser fluencies of 10, 15, and 20. This procedure was repeated using exposure times of 60sec and 90sec. As well as, this process was repeated by exposure with 30 sec, 60 sec and 90 sec exposure times, 10ms and 20ms pulse durations and with different laser fluencies 5, 10, 15 and 20J/cm2, separately. *Results:* A significant reduction (p = <0.0001) in the mean values of the colony was observed with the increase of laser fluency doses compared with control at the same pulse duration. A significant reduction (p = <0.0001) in the mean count of the colonies was observed in the comparison between two laser fluences at the same pulse duration. *In conclusion*, the exposure times, pulse durations and laser fluencies of pulsed alexandrite laser showed an effect on the mean count of the colonies of S aureus bacteria and determined the effective dose.

Keywords: laser, Staphylococcus aureus, Bacteria growth

Introduction

Laser is a novel physical therapy technique used to treat a variety of conditions, including wound healing, inhibition of bacterial growth, and postoperative

wounds[1]. High-power pulsed alexandrite laser therapy is one of the most prevalent forms of laser therapy, which is a noninvasive method for treating various pathological conditions, thereby enhancing functional capacities and quality of life. It is a modern medical and physiotherapeutic technology. Generally, the Alexandrite laser emits infrared light with a wavelength of 755 nm, allowing it to propagate and penetrate tissues[2]. Laser irradiation is effective against Staphylococcus aureus, Streptococcus anginosus, and numerous other types of bacteria[3]. In addition, many studies provide broad information regarding the fungal and bacterial effects of laser therapy utilizing varying energies, wavelengths, and dosages[4]. Laser therapy can eliminate bacteria by altering DNA. In addition, infections' water molecules absorb laser photons, resulting in inhibition or death[5]. Multiple studies have proven that laser therapy has antibacterial effects against Gram-negative and Gram-positive bacteria[6]. Staphylococcus aureus, Gram-positive and a member of the Staphylococcaceae family, the family Staphylococcaceae, is a spherical, grape-shaped bacterium about 1μm in diameter. Staphylococcus aureus is a coexisting disease, often asymptomatic, found on healthy individuals' skin, skin glands, and mucous membranes, including their noses and stomachs[7].

Staphylococcus is the most prevalent cause of human infections and is typically found in the skin and wounds. Staphylococci are primarily responsible for skin infections. Thus, they can transmit disease to healthy members of the population and hospitalized patients[8]. Staphylococcus generally does not cause disease transmission unless it enters the body via damaged skin or mucous membranes or if the immune system is compromised. If these conditions exist, these bacteria will become transmissible and cause mild or severe disease in the community and hospital-acquired illness[9]. Bacteria may colonize several settings, but animals and humans provide the ideal conditions for bacterial growth. The high rate at which S. aureus acquires resistance to numerous antibiotic classes, complicating therapy, is a critical clinical concern linked with this organism. Historically, S. aureus resistance emerged within two years of penicillin's introduction[10]. Pulsed Alexandrite laser has been widely used in many areas of physical therapy due to its practical application and great results [11]. However, few studies have been conducted to explain its effect on bacterial development and the effective dose for killing bacteria has not yet been determined. Therefore, our research focused on the application of a high-power pulsed alexandrite laser in vitro to evaluate the effect of a pulsed alexandrite laser on antibiotic-resistant bacteria utilizing varying exposure times, pulse durations, and laser fluencies to determine which dose is more effective on S. aureus bacteria.

Materials and Methods

Experimental Setups

System Setup

The laser system was fixed vertically on a mechanical jack supported with a height tuner screw on the plane bench; so the laser beam could fall vertically on the test sample, and the laser aperture was stuck to the test sample.

Laser Parameters

The laser that was used in this study was the alexandrite laser which was considered a pulsed laser and had the following parameters: The wavelength was 755 nm, the beam diameter was (14 mm), the exposure times varied (30, 60, 90) seconds, the laser fluency (5, 10, 15 and 20 $J.Cm^{-2}$).

Fluency = Energy (J) /Area (cm^2) = $J.Cm^{-2}$

Where:

Energy is the power of the laser multiplied by pulse width (watt × second).

Area = is the exposed area to the laser beam (cm^2)

Bacterial Samples Preparation

A loopful of the resistant isolates culture was transferred from the nutrient agar slants to a test tube containing 10ml of brain-heart infusion broth, then incubated at 37°C for 18–24 hrs. Serial dilutions were made by tubes containing physiological saline to obtain appropriate CFU. The bacterial broth was compared with McFarland tubes to determine the number of bacteria= 1.5×10^{8} mI/CFU.

Irradiation Procedures: Irradiation was as follows:

The bacteria sample was centrifuged at a speed of (3500rpm) for 6 minutes. The precipitant was kept; the normal saline was added and Centrifuged again. A series of dilutions was made till the solution had Turbidity. (1ml) from the bacterial suspension was taken by a micropipette and placed in a sterile Eppendorf tube. The bacteria sample was exposed to the alexandrite laser with exposure times (30, 60, 90) seconds; the number of bacteria was the same for each dose, which =1.5×10^{8} CFU / ml.

The study was carried out after the bacteria were diagnosed as being antibiotic-resistant. They were exposed to different doses of Alexandrite laser. Three samples of bacteria were exposed to laser beams for 30 seconds with a 5ms pulse duration and with a laser fluency of 5J/cm2, and this process was repeated with laser fluencies of 10, 15, and 20. This procedure was repeated using exposure times of 60sec and 90sec. This process was repeated by exposure with 30 sec, 60 sec and 90 sec exposure times, 10ms pulse duration and laser fluencies 5, 10, 15 and 20J/cm2, separately. Also, the previous process was repeated by exposing the bacteria with different exposure times (30 sec, 60 sec and 90 sec), 20ms pulse duration and with different laser fluencies (5, 10, 15 and 20J/cm2), separately.

Statistical Analysis

One way of variance ANOVA, Least Significant Difference (LSD) and correlation was performed to test whether group variance was significant. Data were expressed as mean± Standard Deviation (S.D.), and Statistical significance was carried out using SPSS program version 26.

Results

The results of the effect of an Alexandrite Laser pulsed on staphylococcus aureus bacteria growth (by the mean values of colony count) will presented in this study. The study was carried out using different exposure times (30, 60 and 90 sec) with different fluencies of an Alexandrite Laser pulse (5,10,15 and 20 J.Cm^{-2}), as well as using different pulse durations (5,10 and 20 ms).

Exposure time 30 sec

		Laser Fluency J.Cm^{-2}			
Pulse duration	**Control Mean ± SE**	**5 Mean ± SE**	**10 Mean ± SE**	**15 Mean ± SE**	**20 Mean ± SE**
P.d= 5ms	216±7.35 a	126.67±2.9 A,b	87.67±4 A,c	74.67±3.6 A,d	41.67±2.3 A,e
P.d=10ms	246±4.18 a	214.67±4.4 B,b	174.67±8.17 B,c	142±6 B,d	80.33±6.17 B,e
P.d=20ms	241±7.12 a	224.67±8.2 B, a	185.33±6.85 C,b	160.67±7.25 B,c	85.67±6.88 B,d

Table 1. The mean values of colony count for experimental samples and control of staphylococcus aureus bacteria after being treated with different fluencies of an Alexandrite Laser pulse (5,10,15 and 20 J.Cm^{-2}) and different pulse durations 5, 10, 20ms) at exposure time 30 sec.

Different small letters (a, b, c, d and e) in the row are significant at $p \leq 0.05$, and the same letters are Non-Significant, SE: Standard error mean. Different capital letters (A, B and C) in the column are significant at $p \leq 0.05$, and the same letters are Non-Significant, SE: Standard error mean.

Statistical analysis of the results of Table 1 shows a reduction in the mean values of colony count after being treated with different fluencies (J.cm^{-2}) of an Alexandrite Laser pulse compared with untreated control at the exact pulse durations (ms). For the 5ms pulse duration, the reduction in the mean values of colony treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} fluencies of an Alexandrite Laser pulse in comparison with the control were by 41%, 59%, 65% and 81%, respectively. Also, for the 10ms pulse duration, the reduction in the mean values of colony count treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} fluencies of

an Alexandrite Laser pulse in comparison with the control were by 13%, 29%, 42% and 67%, respectively. As well as, for the 20ms pulse duration, the reduction in the mean values of colony count of S. aureus bacteria treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} fluencies of an Alexandrite Laser pulse in comparison with the control were by 6%, 23%, 33% and 64%, respectively (Table 1).

According to the results presented in Table 1, the mean values of colony count for control were compared with experiment samples based on different laser fluency doses at one pulse duration and 30 sec exposure time concerning the rows. A significant reduction (p = <0.0001) in the mean values of the colony was observed with the increase of laser fluency doses compared with control at the same pulse duration. A significant reduction (p = <0.0001) in the mean count of the colonies was also observed in comparison between two laser fluencies at the same pulse duration. However, there are no significant differences in mean values of colony count between control and 5 J.cm^{-2} at 20ms pulse duration, as shown in Figure 1.

Concerning different pulse durations (5, 10 and 20ms) at the same fluency effect on bacteria colonies and at 30 sec exposure time, there is an increase in mean values of colony count with increased pulse duration to 10ms and 20ms compared with 5ms pulse duration. A significant difference was ($p \leq 0.05$) noticed in mean values of colony count between pulse durations (5ms and 10ms, 5ms and 20ms). However, there are no significant differences in the mean values of colony count between 10ms and 20ms pulse duration at 5, 10 and 20 J.cm^{-2} laser pulse fluencies, as shown in Figure 1.

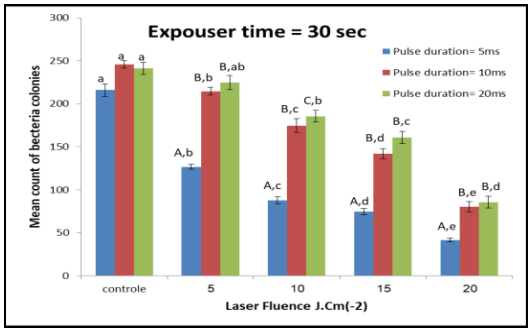

Figure 1. Relationship between the mean of bacteria colonies and four fluencies of an Alexandrite Laser pulsed (5, 10, 15 and 20 J.cm^{-2}) with three pulse durations (5, 10 and 20ms) at 30 sec exposure time.

Exposure time 60 sec

		Laser Fluency J.Cm^{-2}			
Pulse duration	**Control Mean ± SE**	**5 Mean ± SE**	**10 Mean ± SE**	**15 Mean ± SE**	**20 Mean ± SE**
P.d= 5ms	216±7.35 a	116.67±2.96 A,b	80.67±2.4 A,c	70±2.55 A,d	29.67±3.88 A,e
P.d=10ms	246±4.18 a	196.67±3.38 B,b	165.67±6.47 B,c	120.67±8.44 B,d	58.33±5 B,e
P.d=20ms	241±7.12 a	204.67±7.8 B,b	180±3.8 B,c	156±7.52 C,d	78.67±3.9 C,e

Table 2. The mean values of colony count for control and experimental samples of staphylococcus aureus bacteria after being treated with different fluencies of an Alexandrite Laser pulse (5,10,15 and 20 J.Cm^{-2}) and different pulse durations 5, 10, 20ms) at an exposure time 60 sec.

Small letters (a, b, c, d,e) in the row are significant at p ≤ 0.05, and the same letters are Non-Significant, SE: Standard error mean. Different capital letters (A, B) in in column are significant at p ≤ 0.05, and the same letters are Non-Significant, SE: Standard error mean.

From the analysis of the results of Table 2, the mean values of colony count after treated with different fluencies (J.cm^{-2}) of an Alexandrite Laser pulse compared with control at the exact pulse durations (ms) involved in the current study and at exposure time 60 sec. For the 5ms pulse duration, the reduction in the mean values of colony count treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} fluencies of an Alexandrite Laser pulse in comparison with the control were by 46%, 63%, 68% and 86%, respectively. Also, for the 10ms pulse duration, the reduction in the mean values of colony count treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} fluencies of an Alexandrite Laser pulse in comparison with the control of S. aureus were by 20%, 33%, 51% and 76%, respectively As well as, for the 20ms pulse duration, the reduction in the mean values of colony count of S. aureus treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} fluencies of an Alexandrite Laser pulse in comparison with the control were by 15%, 25%, 35% and 67%, respectively (Table 2).

According to the results presented in Table (3.2), the mean values of the control colony count were compared with experiment samples based on different laser fluency doses at one pulse duration and 60 sec exposure time concerning the rows. A significant reduction (p = <0.0001) in the mean of the bacteria colonies was observed with the increase of laser fluency doses at the same pulse duration. As well as a significant reduction (p = <0.0001) in the mean of the bacteria colonies was observed in comparison between two laser energies at the same pulse duration (see Figure 2).

Concerning different pulse durations (5, 10 and 20) at the same laser fluency effect on bacteria colonies and at 60 sec exposure time (respect to the column), there is an increase in the mean of the colonies with increased pulse duration to 10ms and 20ms comparing with 5ms pulse duration. A significant difference was ($p \leq 0.05$) noticed in the mean of the colonies between pulse durations 5ms and 10ms at different fluencies, except at 10 J.cm^{-2}, where the *p*-value was less than 0.05. Significant differences in mean values of colony count between 10ms and 20ms pulse duration were ($p \leq 0.05$) with all laser fluencies except at 5 J.cm^{-2} and 10 J.cm^{-2} were not significant, as shown in Figure 2.

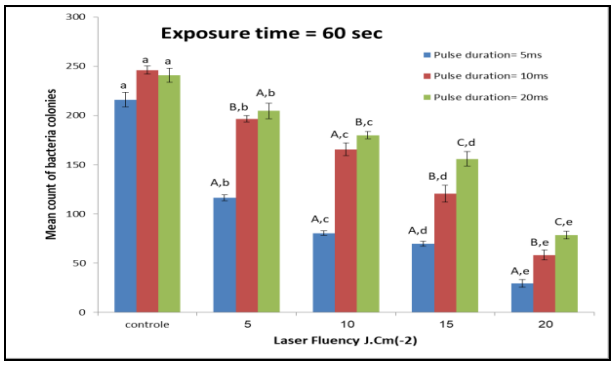

Figure 2. Relationship between the mean of bacteria colonies and four fluencies of an Alexandrite Laser pulsed (5, 10, 15 and 20 J.cm^{-2}) with three pulse durations (5, 10 and 20ms) at 60 sec exposure time.

Exposure time 90 sec

		Laser Fluency J.Cm^{-2}			
Pulse duration	**Control Mean ± SE**	**5 Mean ± SE**	**10 Mean ± SE**	**15 Mean ± SE**	**20 Mean ± SE**
P.d= 5ms	216±7.35 a	99.67±4.17 A,b	74.67±3.5 A,c	54±5.77 A,d	15.67±2.6 A,e
P.d= 10ms	246±4.18 a	162.67±5.18 B,b	140.33±5.86 B,c	90.67±4.76 B,d	38.33±3.45 B,e
P.d= 20ms	241±7.12 a	181.33±3.9 C,b	166.33±3.5 C,c	142±2.34 C,d	55.33±4.48 C,e

Table 3. The mean values of colony count for control and experimental samples of staphylococcus aureus bacteria after being treated with different fluencies of an Alexandrite Laser pulse (5,10,15 and 20 J.Cm^{-2}) and different pulse durations 5, 10, 20ms) at an exposure time 90 sec.

Small letters (a, b, c, d,e) in the row are significant at p ≤ 0.05, and the same letters are Non-Significant, SE: Standard error mean. Different capital letters (A, B) in the column are significant at p ≤ 0.05, and the same letters are Non-Significant, SE: Standard error mean.

From the analysis of the results of (Table 3), there is a reduction in the mean values of colony count after being treated with different fluencies (J.cm^{-2}) of an Alexandrite Laser pulse when in comparison with control of colonies at the exact

pulse durations (ms) involved in the current study and at exposure time 30 sec. For the 5ms pulse duration, the reduction in the mean values of colony treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} fluencies of an Alexandrite Laser pulse in comparison with the control were by 54%, 65%, 75% and 93%, respectively. Also, for the 10ms pulse duration, the reduction in the mean values of the colony treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} fluencies of an Alexandrite Laser pulse in comparison with the control of S. aureus bacteria (untreated) was by 34%, 43%, 56% and 84%, respectively As well as, for the 20ms pulse duration, the reduction in the mean values of colony treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} fluencies of an Alexandrite Laser pulse in comparison with the control of S. aureus bacteria (untreated) were by 25%, 31%, 41% and 77%, respectively.

According to the results presented in (Table 3), the mean values of colony count for control were compared with experiment samples based on different laser fluency doses at one pulse duration and 90 sec exposure time concerning the rows. A highly significant reduction (p = <0.0001) in mean values of colony count was observed with the increase of laser fluency doses at the same pulse duration. As well as, a significant reduction (p = <0.0001) in mean colonies was observed in comparison between two laser fluencies at the same pulse duration, as shown in Figure 3.

Concerning different pulse durations (5, 10 and 20ms) at the same laser fluency effect on bacteria colonies and at 90 sec exposure time (respect to the column), there is a decrease in the mean of the colonies with increased pulse duration to 10ms and 20ms comparing with 5ms pulse duration. A significant difference was ($p \leq 0.05$) noticed in the mean of the bacteria colonies between pulse durations (5ms and 10ms, 5ms and 20ms); see Figure 3.

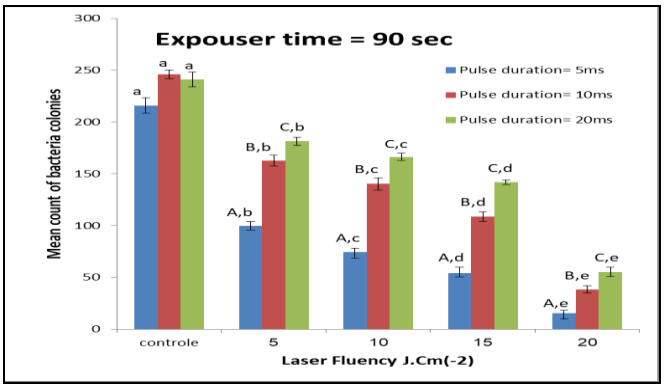

Figure 3. Relationship between the mean of bacteria colonies and four fluencies of an Alexandrite Laser pulsed (5, 10, 15 and 20 J.cm^{-2}) with three pulse durations (5, 10 and 20ms) at 90 sec exposure time.

Discussion

Over the past few years, studies using various types of lasers with varying wavelengths and energies for therapy have yielded positive effects and outcomes in laboratory studies and clinical practices to enhance wound healing, treat inflamma-

tion-infected wounds, and inhibit bacterial and fungal growth[3,12]. However, the development of high-energy pulsed alexandrite lasers with a wavelength of 755 nm and new optical systems has resulted in its widespread application in numerous fields of medicine and physical therapy, such as an antimicrobial, to reduce or eliminate disease-causing organisms and numerous types of bacterial-infected wounds [1,13].

I am using a laser with specific parameters such as wavelength, exposure time, pulse duration, and laser fluency, which results in shrinkage of the bacterial cell and deoxyribonucleic acid (DNA), which modifies gene expression of bacteria and inhibits bacterial growth and activity. In addition, laser light immediately affects cell integrity, preventing cell division and increasing the number of metabolically inactive cells[3].

Monochromic is one of the fundamental laser mechanisms that modifies the function of cells and tissues depending on the property of the light. (e.g., wavelength, coherence)[14], this enables effective coupling to chromophores' maximum absorption, allowing for maximum photoactivation and stimulation of biological activities[15].

The purpose of the current study is to assess the impact of a high-power alexandrite laser on the in vitro growth of S. aureus, and the main result of the study is that a high-power alexandrite laser led to a reduction in the growth of experimental S. aureus relative to the control S. aureus. Irradiation with a high-power alexandrite laser resulted in a reduction in the tested bacteria. This result indicates that using a 755nm wavelength can reduce the total number of bacteria measured by the colony counting method.

The results of the current study indicated a highly significant difference between laser fluency and control when fixed exposure time and pulse duration; there is a reduction in the mean values of colony count for experimental samples compared with control when increasing the laser fluencies. As well as, there is a reduction in the mean values of colony count for experimental samples comparison with control when decreasing the pulse duration of the laser. The slight and slow light absorption by chromophore increases the oxidative reaction of mitochondria and adenosine triphosphate (ATP), ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) production by photochemical effects, leading to a tissue stimulation phenomenon "photobiology effect" that kills bacteria[14]. In contrast, in the processes performed on bacteria, the laser light is significantly absorbed by the material to which the bacteria adhere, resulting in a sufficiently high temperature in the exact location to kill the attached organisms[16].

Exposure time to the laser represents a radiant energy dose; the longer the time of irradiation, the higher the dose. The dose of laser 30 sec exposure time,5ms pulse duration with 20 J/cm2 laser fluency was more effective in the S. aureus count than in all doses of 5, 10 and 15 J/cm2 laser fluency. Also, the dose of laser 60 sec exposure time,5ms pulse duration with 20 J/cm2 laser fluency was more effective in the S. aureus count than in all doses of 5, 10 and 15 J/cm2 laser fluencies. This process applies with a 90-second exposure time. Therefore, exposure

time and laser dose (pulse duration and laser fluency) are two factors that determine the effective dose of a pulsed alexandrite laser when calculating colonies, and more exposure time combined with a higher dose may be required to obtain the best results[17]. Additional exposure may increase the photo-thermal effects of the laser on bacteria.

Conclusion

Exposure times, pulse durations and laser fluencies of pulsed alexandrite laser show an effect on of mean count of the colonies of S aureus bacteria and determined effective dose depending on Increasing the exposure time leads to increased bacterial killing when laser fluency and pulse time are fixed. Increasing the laser fluency leads to increased bacterial killing when exposure time and pulse time are fixed. Decreasing the pulse duration increases bacterial killing when exposure time and laser fluency are fixed.

Recommendation

High-power pulsed alexandrite laser light at specific wavelengths (755nm) and varying exposure times, pulse durations, and laser fluencies might positively affect the reduction of S. aureus growth. Nonetheless, clinical research should be undertaken. Laser irradiation has numerous advantages, including a reduced treatment cost, a shorter treatment time, few side effects, and an alternative to the administration of antibiotics systemically. A pulsed, high-power alexandrite laser can effectively inhibit the growth of S. aureus in infected wounds.

Acknowledgments

The authors thank the University of Baghdad, College of Medicine. Special thanks go to Baghdad Teaching Hospital, Medical City, Baghdad, for his support and advice during this work.

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Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023
Citation: Mustafa, E.A.; Dawood, N.S.; Mohammed, K.I.A. Study the effect of the different doses from the laser on Staphylococcus aureus Bacteria growth in vitro. Revis Bionatura 2023;8 (3) 74. http://dx.doi.org/10.21931/RB/CSS/2023.08.03.74