Published Online First: January, 2024
https://doi.org/10.21123/bsj.2024.9041
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Antibacterial Efficacy of 940 nm Diode Laser against Cariogenic Bacteria (Tooth Decay-causing Bacteria)

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Received 08/05/2023, Revised 10/07/2023, Accepted 12/07/2023, Published Online First 20/01/2024

Abstract

Dental caries is an extremely prevalent infectious disease caused by gram-positive bacteria mainly streptococcus mutans which is considered the major etiological factor causing dental caries. In recent years, there has been an enormous increase in the use of laser technology in medicine and dentistry and it has been demonstrated to have a considerable antibacterial action with no harm to the oral tissues. The study goal was to assess a diode laser's effectiveness as an antibacterial agent against the bacterium species S. mutans at various doses. The study was performed on streptococcus mutans microorganism at 106 CFU/ml concentration which received irradiation from a diode laser with 940 nm a central wavelength to investigate three output powers (1 watt, 2 watts, and 3 watts) for 30 s exposure time, and chlorohexidine (CHX) was used as a positive control group. Bacterial growth (CFU/ml) was calculated 24 hours after laser exposure. A significant diminish in CFUs/ml of S. mutans bacteria was observed 24 hours following irradiation by the three different powers. The result showed a statistical difference (p-value < 0.01) as compared to the negative control group without treatment. The current study demonstrated that the 940 nm diode laser was successful and efficient in the reduction of S. mutans growth at different doses.

Keywords: Bactericidal effect, Chlorhexidine (CHX), Dental caries, Laser irradiation, Streptococcus mutans.

Introduction

Streptococcus mutans, a gram-positive, facultative anaerobic cocci that is typically found in the mouth is also non-motile, non-spore-forming, and catalase-negative [1]. It is the main etiological factor of dental caries, which affects 95% of people of all ages worldwide [2]. There are many factors that enable *S.mutans* to cause dental caries, including adhesion to enamel surfaces, formation of acidic metabolites, the capability to accumulate glycogen reserves, and synthesis of extracellular polysaccharides [3]. Mutans streptococci create a lot of acids, which makes the environment acidic and increases the risk of cavities also it has the ability to survive in an acidic medium (greater tolerance to low pH) [1]. This acidic state results from sugar fermentation by cariogenic microorganisms that contribute to dental plaque buildup and the development of biofilms [4]. Dental caries is a multifactorial, chronic dynamic disease, that is mediated by biofilms and sugar and involves the presence of cariogenic bacteria [5]. Bacterial plaque, fermentable substrate (sugar), vulnerable tooth surfaces, and time, into which dental hard tissues undergo phasic demineralization by acid produced from food fermentation by bacteria [6]. It is

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becoming common practice among dentists all over the world to use antibacterial agents which can aid in lowering the prevalence of pathogenic bacteria [7]. The majority of studies presented findings related to the use of cavity disinfectants like chlorhexidine, laser systems, and sodium hypochlorite (NaOCl). In some of the studies, additional disinfectant solutions were also assessed [8]. There are numerous techniques to lower the prevalence of dental caries, such as fluoride and chemical antibacterial agents, although they are not always highly efficient and may have negative side effects [9, 10] or the bacteria may develop a resistance to this antibacterial agent, there has been an obvious increase in the percentage of bacterial pathogens that are resistant to numerous antibacterial agents [11] due to inappropriate utilization and widespread consumption of antibiotics [12]. As a result, various antibacterial methods have a good bactericidal effect and do not have any negative side effects are required such as lasers. Rapid advancements in laser technology (including wavelengths, methods, and delivery systems) have allowed for its usage in a wide range of fields, including medicine, physics, biology, biotechnology, biochemistry, and dentistry [13]. It has been demonstrated that a variety of lasers, including diode lasers, Er-YAG, and Nd-YAG, exhibit bactericidal properties [14]. Because of its low cost, portable, effective bactericidal action through its thermal effect, and temperature rise that is within a safe range for permanent teeth, the diode laser has gained popularity recently and is now commonplace in dental offices [15]. In addition, diode laser irradiation can reach into the dentinal tubules up to 1000 μm from the surface while the chemical disinfectants have a penetrating depth of only 100 μm, so this makes it potent for disinfection [16]. Also, the effectiveness of Er,Cr:YSGG lasers in terms of bacterial elimination has attracted a lot of study interest, as the bacteria's water molecules make them an ideal target for this laser, which causes bacterial destruction when energy absorption happens [17].

Laser treatment is recognized as an effective method thanks to many benefits, including a reduction in the bacterial count. Diode lasers, in particular, have been demonstrated in studies to be successful at reducing bacterial counts and eliminating microorganisms through their photothermal effect, hence they are utilized for disinfection[18]. Also, the effectiveness of Er,Cr:YSGG lasers in terms of bacterial elimination has attracted a lot of study interest, as the bacteria's water molecules make them an ideal target for the Er,Cr,YSGG laser, which causes destruction when the laser energy is absorbed[19].

Photothermal interaction is a reaction that occurs when a laser beam strikes a tissue and then the light energy is converted into heat through a thermal interaction with the cellular molecules. It develops several impacts on tissue like hyperthermia, coagulation, vaporization, carbonization, and finally melting[20].

Theoretically, laser energy might kill bacteria directly by disrupting their cell membranes and killing them as a result of the production of reactive oxygen species brought on by the strong absorption of laser light[21]. It has been hypothesized that the inhibition of bacterial growth after exposure to laser light has resulted from the effect of free radicals on membrane lipids and DNA[22]. Free radicals are extremely reactive chemical substances that can interact with bacterial cell molecules including membrane lipids, proteins, and nucleic acids. This is going to damage the bacterial plasma membrane, massive vacuole development, leak cytoplasmic material, and complete cell distraction[23].

The aim of this study is to evaluate the antibacterial effectiveness of a diode laser with 940 nm wavelength on the viability of *S. mutans* bacteria and compare this antibacterial effect (if ever found) with that of chlorohexidine.

Materials and Methods

Selection of Bacterial Species

As previously mentioned, dental caries is thought to be mainly caused by *Streptococcus mutans (S. mutans)* according to numerous epidemiological, experimental, and animal research[24].This pathogenic bacterium promotes rapid carbohydrate digestion and produces an acidic environment in the oral cavity which results in tooth demineralization and caries[25]. This bacterium is a member of a group of acidogenic and aciduric Gram-positive bacterial species and is most prevalent in humans, comprising the specific microorganisms that have long been the subject of research into how caries begins and progresses [26].

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Samples Collection, Isolation & Identification of Mutans Streptococci Bacteria

Plaque and saliva samples were collected from 30 patients with dental caries between the ages of 15 and 40 years of both sexes, non-smokers and without any systemic disease, who visited the dental clinics of the College of Dentistry/University of Baghdad. The samples were collected by swabs using sterile wet transport media and then it is transported by ice box to the Ministry of Science and Technology/Food Contamination Research Center Laboratories. 100 microliters from the collected samples were cultured on the mitis salivarius bacitracin agar MSBA selective medium, which prevents the growth of all bacterium types except *S. mutans*, *S. salivarius*, and *S. oralis* as shown in Fig. 1. The isolated colonies from the sample culture on the MSBA plate were cultured in an anaerobic jar for 24 hours at 37-celsius degrees with 5-10% CO2. The isolated bacteria were identified by the conventional method which involved microscopic examination and biochemical tests and the final identification was done by polymerase chain reaction (PCR).

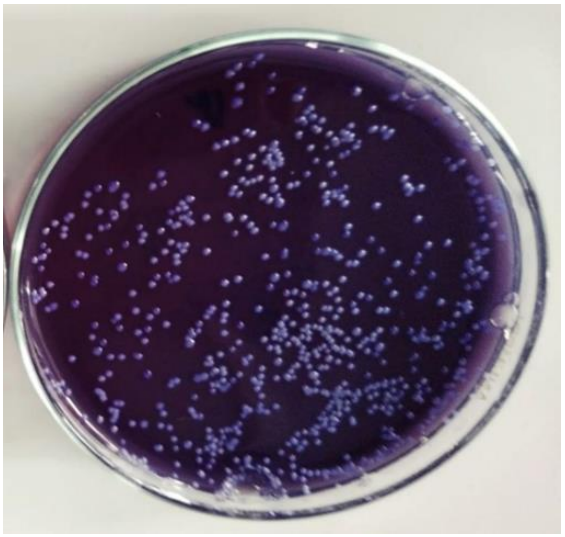

Figure 1. S. mutans growth on MSBA after 24 hours at 37°C.

Bacterial Samples Preparation

Streptococcus mutans was the bacteria used in this study. It was cultivated in brain heart infusion broth (HIMEDIA, India) at 37°C for 24 hours with turbidity adjusted to 0.5 scale McFarland (1 ml of 0.5 McFarland usually contains 10^8 bacteria), and 10-fold dilution to 0.5 McFarland suspension was decided to make to achieve a concentration of 5x 10^6 bacteria in 1 ml for easy counting them.

Laser Irradiation

Diode laser (Epic, Biolase, USA) with three output powers 1 W, 2 W, and 3 W in continuous mode for 30 s exposure time provided by a 200 µm fiber tip diameter (E2-20, Biolase, USA) was embedded into the sterile Eppendorf tubes containing 1 ml of bacterial suspension (5x 106 cells/ml) in a circular movement continuously to make sure laser distribution uniformly in the suspension volume as represented in fig. 2. After each use, 70% ethyl alcohol was used to sterilize the laser tip[27]. The irradiated suspension and control were grown on MSBA overnight at 37℃

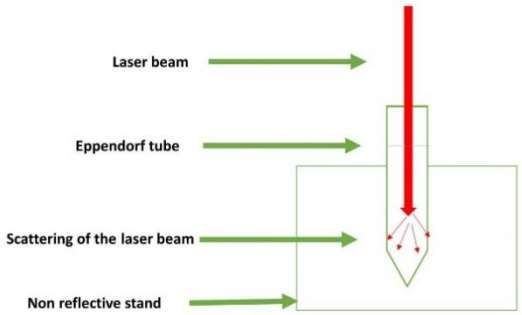

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Figure 2. Arrangement of laser irradiation

Experimental Groups

50 samples are divided into five experimental groups, each group having ten samples (n=10)

Group A: Negative control group bacterial suspension containing (10^6 CFU/ml) bacterial concentration without any treatment.

Group B: Positive control group into which 2% CHX was used to treat the bacteria.

Group C: Irradiation of bacterial suspension by diode laser 940 nm 1 W output power, CW, 30 s exposure time.

Group D: Irradiation of bacterial suspension by diode laser 940 nm 2 W output power, CW, 30 s exposure time.

Group E: Irradiation of bacterial suspension by diode laser 940 nm 3 W output power, CW, 30 s exposure time.

Antibacterial Activity Determination

The study's objective is to assess the antibacterial properties, so the reduction in bacterial number

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following exposure to different antibacterial treatments was the main focus of this study and to check how effective the treatment is. After treating all the samples of bacteria with different treatment modalities, bacterial counting using CFU/ml was made by taking a part of the bacterial suspension and streaking it on a bacterial growth selective media (MSBA), cultured for 24 hours after being serially diluted by 3-dilution fold 10^{-1}, 10^{-2}, and 10^{-3}. By using the following equation, the number of CFU that were counted on the MSA plates was determined per milliliter of the initial sample:
The number of CFU/ml = Number of CFU x Dilution factor[28, 29].

Results and discussion

For each group, the mean and standard deviation values were calculated**,** and the data were processed by a one-way analysis of variance test (ANOVA) to compare the means of different groups, Table 1. Outcomes were presented as mean and SD, p values less than or equal to 0.05 denoting statistical insignificance while when it is greater or equal to 0.05, 0.01, and 0.001 denoting statistically significant differences. The statistical analysis was finished by using SPSS (v 20).

Table 1. Descriptive statistics of all study groups after antibacterial treatment

Group order	Group type	Mean	SD	LSD	P value
Negative control group		500 x10^4	250	E	
Group A	Positive control (CHX)	34 x10^4	5.8	B	0.001
Group B	Diode laser 1 Watt	24 x10^4	3.6	A	0.001
Group C	Diode laser 2 Watt	139 x10^4	6.9	C	0.001
Group D	Diode laser 3 Watt	230 x10^4	4.5	D	0.001

The results are expressed by the mean and standard deviation of CFU, there is a significant decrease in colony counts for all groups after interventions. The negative control group which represents the bacteria without treatment has the highest mean value, followed by group D, group C, group A, and finally, group B has the lowest mean value. The highest kill rate of Group B 1W (24 x104) than Group C 2W (139 x104) and Group D 3W (230 x104) for all the ten samples of each group.

The letters A, B, C, D, and E represent the various degrees of significance. The significant differences between the tested means were ascertained using the LSD test. Highly significant starting with the letter (A) and ending with the letter (E). The maximum reduction in the number of colonies forming unit of bacteria was achieved by group B, while the minimum reduction was obtained by group D as represented by Fig. 3.

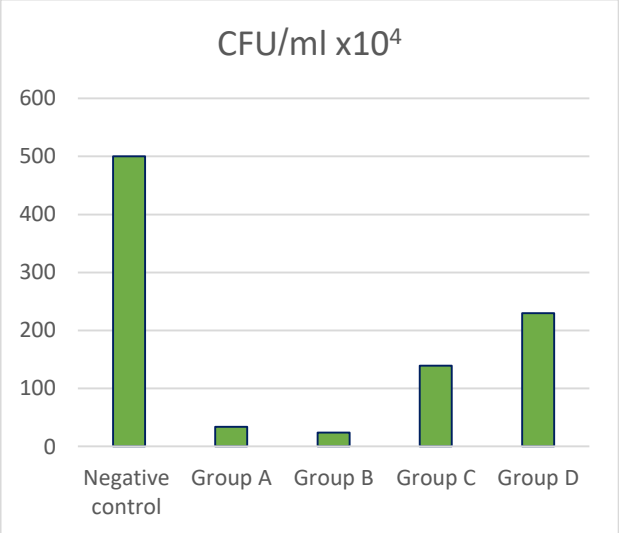

Figure 3. Graphical representation of antibacterial activity among the tested groups.

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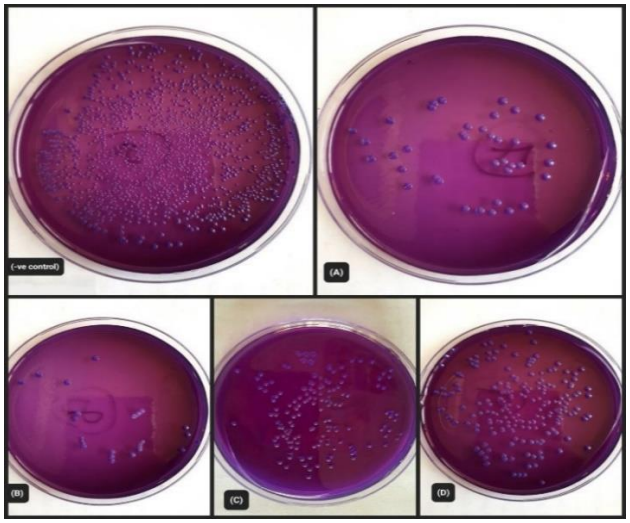

Figure 4. Streptococcus mutans growth of all experimental groups (-ve control- no treatment, A: CHX, B: 1 Watt, C: 2 Watt, and D: 3 Watt).

One of the primary goals of restorative treatment is to restore function and aesthetics while minimizing or completely eliminating bacteria in and around the filling to assure the treatment's success and decrease the risk of developing secondary caries and treatment failure.

The objective of the current investigation was to assess the effect of the 940 nm diode laser antibacterial property on the survival of the *S. mutans* microorganisms. The study's findings indicated that there was a decrease in the number of bacterial colonies and a statistical difference between the CFU of the bacteria before and after irradiation (high significance P value 0.001). It is worth mentioning that this type of laser therapy is considered a HLLT as the powers used are 1 watt, 2 watts, and 3 watts.

Diode lasers have been shown in numerous studies to be able to remove layers generated by bacteria and enhance the efficacy of tooth restoration[4]. The photo-disruptive and thermal effects of the diode laser's irradiation, which caused sub-lethal damage to the bacteria, can be linked to the antimicrobial action proposed when it was utilized[30]. If done properly, laser irradiation is known to cause bacterial cell wall disintegration, bacterial integrity disturbance, a buildup of denatured proteins, cell lysis, and ultimately microbial death. Additionally, the diode laser's near-infrared light can directly destroy bacteria that are pigmented and contain protoporphyrin IX, strengthening disinfection[31].

Hendi et al study on the antibacterial effects of 940 nm diode laser on *E. faecalis* bacteria employed the laser parameters of 1 W output power and (45 s) exposure period of 15 s for three separate exposures. The results showed a reduction in bacterial colonies between the time they were exposed to 940 nm diode laser light and before exposure (P value 0.001), which agreed with the findings of the current investigation[32].

Numerous studies were conducted to determine the effects of lasers on the growth of *Streptococcus mutans*. As an example, in a study by Robati et al[4]., *Streptococcus mutans* and *Lactobacillus* bacteria at 10^8 CFU/ml concentration were irradiated by a diode laser source with a central wavelength of 980 nm in order to assess the effectiveness of the laser using different doses and times. The results revealed that the 980 nm diode laser is particularly successful at inhibiting the growth of the two types of bacteria at various times and doses 24 hours after the irradiation[4] and this is significantly related to the findings of the current study because the laser action was examined 24 hours after exposure.

In Castelo et al study[33], *E. faecalis* was eliminated using a 940-nm diode laser, they reported a 70% rate of bacterial elimination by applying laser output was 3.5 watts, and in pulsed mode for 1 minute exposure time. While the current study, laser radiation of bacterial suspension was done in a continuous mode for 30 s irradiation time, so more bacterial colonies were eradicated than Castelo et al investigation, and this agreed with the results of our study as the outpower 1 watt has strongest bacterial killing than the powers 2 watts and 3 watts.

Chemical disinfection using chlorhexidine is one of the most often used disinfecting techniques in dentistry for preventing and reducing the growth of microbes, particularly *S. mutans*[34]. Given that it is a disinfectant that is readily available on the market, the standard 2% CHX was used in this study as a positive control. The study's findings showed that 2% CHX was effective against the *S. mutans* bacterium and had good antibacterial characteristics, as evidenced by a reduction in CFUs/ml that gave a statistically significant difference (P value 0.001) when compared to the control negative group.

The efficiency of the disinfectant is determined by the process of CHX's adherence to microbe cell walls, which causes intracellular component leakage. At low concentrations, CHX's bacteriostatic activity results in the release of the microorganism's tiny molecular weight

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components; However, at higher concentrations, CHX causes cytoplasmic precipitation and/or coagulation, which is most likely caused by protein cross-linkage and exhibits the bactericidal effect[34].

According to the Hassaballah et al study, which compared the effectiveness of grape seed extract, CHX, and laser diode as primary disinfectants for tooth cavities, the diode laser system is a more potent disinfectant for caries lesions than CHX and Grape Seed Extract[35]. In light of the fact that the diode laser group's results are better than those of the CHX group with a static difference (P value 0.001), there is ample agreement with the findings of our current investigation.

Conclusion

From this work results, it is concluded that the application of the diode laser is a successful and effective technology for the elimination of *S. mutans* bacterial colonies at different exposure powers and it can be used as a potent disinfectant in dental clinics by all dentists to help in reducing the incidence of secondary tooth caries by reducing the number of tooth decay bacteria.

Author's Declaration

- Conflicts of interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and images, which are not ours, have been given permission for re-publication and attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee of the Institute of Laser for Postgraduate Studies.

Authors' Contributions

N. M. J. and H. J. T. contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript.

Authorship contribution statement

N. M. J. Conceptualization; N. M. J. Data curation; H. J. T. Formal analysis; N. M. J. Funding acquisition; N. M. J. Investigation; N. M. J. Methodology; H. J. T. Project administration; H. J. T. Supervision; H. J. T. Validation; N. M. J. Visualization; N. M. J. Roles/Writing - original draft; N. M. J. Writing - review &editing.

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الفعالية المضادة للبكتيريا لليزر الصمام الثنائي 940 نانومتر ضد البكتيريا المسببة لتسوس الاسنان

نها محمد جميل، حنان جعفر طاهر

معهد الليزر للدراسات العليا، جامعة بغداد، العراق.

الخلاصة

تسوس الأسنان هو مرض معدي منتشر للغاية تسببه بكتيريا موجبة بشكل رئيسي المكورات العقدية الطافرة والتي تعتبر العامل المسبب الرئيسي لتسوس الأسنان. في السنوات الأخيرة ، كانت هناك زيادة هائلة في استخدام تقنية الليزر في الطب وطب الأسنان ، وقد ثبت أن لها تأثيرًا كبيرًا مضادًا للبكتيريا دون أي ضرر لأنسجة الفم. كان هدف الدراسة هو تقييم فعالية ليزر الصمام الثنائي كعامل مضاد للجراثيم ضد بكتيريا بجرعات مختلفة. تم اجراء الدراسة على بكتريا المكورات العقدية بتركيز106 مستعمرة لكل مليلتر والتي تلقت اشعاع ليزر من ليزر ثنائي الصمام ذي الطول الموجي 940 نانو متر لفحص ثلاثة قدرات مختلفة (1 واط، 2 واط، و 3 واط) لمدة 30 ثانية من وقت التعرض، وتم استخدام الكلوروهيكسيدين كمجموعة تحكم ايجابية. تم حساب النمو البكتيري بعد 24 ساعة من التعرض لليزر.النتائج اظهرت فرق احصائي عالي بالمقارنة مع مجموعة التحكم السلبية بدون معالجة. كإستنتاج، الدراسة الحالية وضحت ان ليزر الصمام الثنائي 940 نانومتر كان ناجا وفعالا في تقليل نمو بكتيريا المكورات العقدية وبجرعات مختلفة.

الكلمات المفتاحية: تأثير مبيد للجراثيم، كلوروهيكسيدين، تسوس الاسنان، التشعيع بالليزر، المكورات العقدية الطافرة.