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Research Article

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In vitro study of the antibacterial property of 940 nm diode laser against Streptococcus mutans bacteria isolated from dental caries

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Abstract

Background: The main etiological element of dental caries is Streptococcus mutans (S. mutans) bacteria, so getting rid of this bacterium has a significant impact on how well restorative treatment goes. New approaches for eradicating bacteria in dentistry have been developed like lasers, metallic nanoparticles, and bioactive materials.
Aim of the study: This study's goal was to assess a diode laser's effectiveness as an antibacterial agent and then compare it with the antibacterial effect of chlorhexidine (CHX) against S. mutans bacteria.
Material and Method: The study was performed by using S. mutans microorganisms collected from patients with dental caries, then a bacterial suspension was prepared at a concentration of 106 CFU /ml and placed in an Eppendorf tube to be treated with various antibacterial modalities, the 30 samples were divided up into three experimental groups: Group I: Negative control group; Group II: Positive control group using 2% chlorhexidine; Group III: Irradiation with diode laser (1 watt output power for 30s exposure time). The number of colony-forming units (CFU) was counted for each group after 24 h of incubation on Mitis Salivarius Bacitracin agar (MSBA) plates.
Results: A significant reduction in the CFUs/ml of S. mutans bacteria was observed 24 hours following treatment by the two approaches (The diode laser and CHX). The findings of the study indicate a significant statistical difference (p-value < 0.01) between the two groups in comparison to the negative control group that did not receive any treatment. Furthermore, the group treated with the diode laser exhibited the greatest drop in bacterial count compared to the group treated with CHX.
Conclusion: Both diode laser and CHX have a good bactericidal effect, but the diode laser had an antibacterial effect superior to CHX. The diode laser was a successful and effective approach for eliminating bacteria and it can be employed as a step in the teeth restoration process.

Keywords: Chlorohexidine, Dental caries, Diode Laser, Disinfection, Laser irradiation.

1. Introduction

Dental caries is a complex, multifactorial, chronic, and dynamic disease that affects 95% of the population of all ages worldwide.(Sadony and Abozaid, 2020) It is mediated by biofilms and sugar in the presence of cariogenic bacteria (Qiu et al., 2020), in which dental hard tissues undergo phasic demineralization by acid produced through food fermentation by bacteria (Pitts et al., 2017). Gram-positive, facultative anaerobic streptococcus mutans is frequently found in the mouth is the main contributory factor to dental caries (Jassim, 2022). The main goal of dental restoration is to remove infected carious tissues and bacteria and replace them with a filling material in order to protect and preserve the remaining tooth structure. However, secondary caries is one of the main causes of restorative failure because it can develop if infected tissues, bacteria, and germs are not completely removed. (Selivany et al., 2020) There are many methods to reduce the occurrence of dental caries, including fluoride and chemical antibacterial agents (Liao et al., 2017; Shallal and Ahmed, 2022) but they are not usually very effective and may have unfavorable side effects, or the bacteria could become resistant to this antibiotic. (Toma and Aziz, 2023) As a result, various antibacterial techniques like lasers that have a strong bactericidal impact with no harmful side effects are needed. Using antibacterial treatments to help reduce the prevalence of infection-causing bacteria is a practice that dentists all around the world are starting to use. (Chalisha et al., 2021) Results from most research were related to the use of cavity disinfectants like chlorhexidine, laser technology, and sodium hypochlorite (NaOCl). Additional disinfection options were also evaluated in some studies. (Coelho et al., 2020)

Because of its antibacterial Efficiency, which includes those against S.mutans, and its antiplaque action, chlorhexidine has been widely utilized in dentistry. (Kandaswamy et al., 2018, Haydari et al., 2017) The majority of authors assessed chlorhexidine's effectiveness as a cavity disinfectant, and according to a number of findings, chlorhexidine is the most widely agreed-upon cavity disinfectant for use in clinical practice (Coelho et al., 2021). When chlorhexidine (CHX) was utilized prior to the application of adhesives, a reduction in residual microbiological contamination and enhancement of the seal of restoration were noted. (Ebrahimi et al., 2018) Many researchers have looked into the various capabilities and characteristics of CHX, including its capability to inhibit matrix metalloproteinases (MMPs) when used before the application of adhesive systems. (Mohammadi et al., 2020)

Rapid developments in laser technology (including wavelengths, techniques, and delivery systems) have made it possible to use it in a variety of disciplines, such as dentistry, physics, biology, biotechnology, and biochemistry. (Saleh et al., 2023) Numerous lasers, including diode lasers, Er-YAG, and Nd-YAG, have been shown to have bactericidal effects. (Wang et al., 2018) The diode laser has lately acquired popularity and is now prevalent in dental offices due to its low cost, portability, efficient bactericidal action through its thermal effect, and temperature rise that is within an acceptable range for permanent teeth (Bahrololoomi et al., 2017). Furthermore, whereas conventional disinfectants only penetrate a depth of 100 μm into the dentinal tubules, diode laser light can do so up to 1000 μm from the surface, making it an effective disinfectant. (Saafan et al., 2018). Also, there has also been a lot of interest in the efficacy of Er,Cr:YSGG lasers in eliminating bacteria since bacteria's water molecules make them a good target for Er,Cr:YSGG lasers, which destroy bacteria when energy is absorbed. (Tokuc et al., 2019)

This study's objective is to assess the antibacterial efficacy of a diode laser with a 940 nm wavelength on the survival of S. mutans bacteria and compare that antibacterial impact to that of chlorohexidine.

2. Material and Method

2.1. Samples Collection, Isolation, and Identification of S. mutans Bacteria

The plaque and saliva samples were obtained from patients with dental caries who visited the dental clinics at the College of Dentistry/University of Baghdad. The samples were obtained using sterile wet transport media, and they were later delivered by ice box to the laboratory. a total of 100 microliters of the obtained samples were grown for 24 h at 37o C on a plate of mitis salivarius bacitracin agar MSBA selective medium. To identify the isolated bacteria, The initial identification of S. mutans was done by conventional methods, which included microscopic examination and biochemical testing, while the final identification was carried

out using the more reliable polymerase chain reaction (PCR) method of detection in epidemiological studies.

2.2. Bacterial sample preparation

A single colony of S. mutans bacteria was taken and cultivated for 24 h at 37o C in BHI broth (HIMEDIA, India). The concentration was modified to 0.5 scale McFarland (1 ml of 0.5 McFarland containing nearly 108 bacteria). A 10-fold dilution to 0.5 McFarland suspension was done to achieve a concentration of 5×106 bacteria in 1 ml to reduce the number of bacterial colonies to be able to count them (Kasraei et al., 2014).

2.3. Laser irradiation

The bacterial solution was exposed to a 940 nm-wavelength diode laser (Epic, Biolase, USA). The output power was 1 W in continuous mode for 30 s of exposure time, delivered by a 200 µm fiber tip (E2-20, Biolase, USA). It was inserted into the sterile Eppendorf tubes containing 1 ml of bacterial suspension (5 × 10^6 cells/ml) with a spiral motion continuously in a clockwise direction from the bottom to the top of the tube, as shown in Figure 1. This ensures laser distribution evenly throughout the entire suspension volume. The laser tip was disinfected with 70% ethyl alcohol after each use. (Sadony and Montasser, 2019) The irradiated suspensions were cultured on MSBA overnight at 37°C.

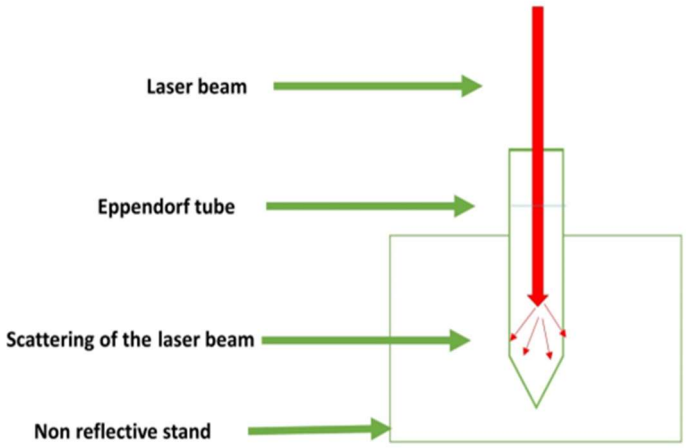

Fig.1: Laser arrangement of bacterial irradiation in the Eppendorf tube.

2.4. Experimental groups

Samples are divided into three experimental groups, each group having ten samples (n=10)
Group I: Negative control group bacterial suspension containing (106 CFU/ml) bacterial concentration without any treatment.
Group II: Positive control group into which 2% CHX
(CERKAMED, Poland) irrigation was used to treat the bacteria.
Group III: Irradiation of bacterial suspension by diode laser 940 nm 1 W output power, CW, 30 s exposure time.

2.5. Antibacterial activity determination

To assess the antibacterial activity, the reduction in bacterial number following exposure to different antibacterial treatments was the main focus of this study, as was checking how effective the treatment is. After treating all the samples of bacteria with different treatment modalities, bacterial counting using CFU/ml was done. This was achieved by taking a portion of the bacterial suspension (100 microlitres) and spreading it on bacterial growth selective media (MSBA) and cultured for 24 h after being serially diluted by 3-dilution folds 10l, 10-2, and 10-3. By using the following equation, the number of CFU was counted on the MSBA plates and determined per millilitre of the initial sample:
Number of CFU/ml = number of CFU x dilution factor (De Mandal and Passari, 2021, Buraihi and Alkurtas, 2020). Moreover, the following equation was used for calculating the killing percentage:
Percentage of killing = 100% – (CFU of the tested group/CFU of the control group) x 100% (Lee et al., 2006).

3. Results

The statistical analysis was completed by using SPSS (v 20). In order to process the data, a one-way analysis of variance (ANOVA) test was employed. Comparing several groups' means. Results were presented as mean and standard deviation (SD), with P values that are higher than 0.05 being statistically non-significant and P values that are less than 0.05, 0.01, and 0.001 being statistically significantly different. The level of significance between the tested means was ascertained using the LSD test represented by the letters from (A) to (C) in decreasing order. The results are summarized in Table 1.

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

Group order	Group type	Mean (CFU/ml)	SD	LSD	P value
Group I	Negative control	500 x10^4	250	C	
Group II	Positive control (CHX)	34 x10^4	5.8	B	0.001
Group III	Diode laser	24 x10^4	3.6	A	0.001

SD = Standard Deviation, LSD = The significance level, P value = probability value.

The results are represented by the mean and standard deviation of CFU following the interventions; colony counts for both groups significantly decreased. Group III has the lowest mean value (24 x 10^4 CFU/ ml), followed by group II (34 x10^4 CFU/ ml). The bacteria untreated in the negative control group, which has the greatest mean value as represented graphically in Figure 2. A, B, and C letters indicate the different levels of significance. The LSD test was used to determine the differences between the tested means that were significantly different. Also, the percentage of killing of bacteria was presented in Table.2

Table 2. Percentage of killing of the study groups.

Tested group	Percentage of killing
Group I	0 %
Group II	99.32 %
Group III	99.52 %

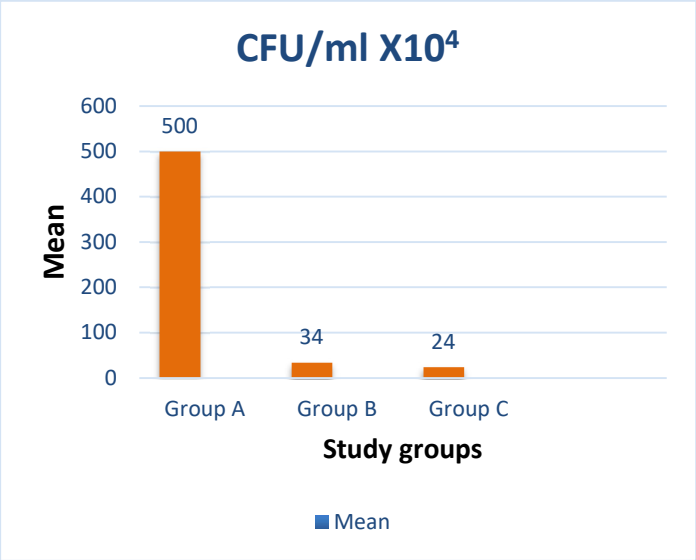

Fig. 2: Graphical representation of antibacterial activity among the tested groups.

Fig. 3: CFU of *S. mutans* bacteria on MSBA plates. Group A: Negative control (no treatment), Group B: Positive control (2% CHX irrigation), and Group C: Diode laser.

4. Discussion

The focus of the present study was to determine how the antibacterial properties of the 940 nm diode laser affected the survival of the S. mutans organisms. According to the results of the research, there were fewer bacterial colonies present, and there was a statistically significant difference between the CFU of the bacteria prior to and following irradiation (high significance P value 0.001). These results agree with a study performed by Robati et al. Streptococcus mutans and Lactobacillus bacteria at 108 CFU/ml concentrations were exposed to diode laser radiation at various doses and exposure durations to evaluate the laser's efficacy. The findings showed that the (980 nm) diode laser is especially effective at preventing the growth of the two types of bacteria at different times and doses 24 h after the irradiation (Robati et al., 2022), and this is strongly in line with the findings of the current study since the laser action was examined 24 h after exposure. This antibacterial action can be attributed to the photo-disruptive and thermal effects

of the diode laser's irradiation, which caused bacterial damage. (Nammour et al., 2021) When laser irradiation is done correctly, it is known to destroy bacterial cell walls, disrupt bacterial integrity, accumulate denatured proteins, cause cell lysis, and finally kill microorganisms. Additionally, protoporphyrin IX-containing and pigmented bacteria can be immediately destroyed by the diode laser's near-infrared light. (El Mobadder et al., 2022). A study by Hendi et al. used a laser with a 1 W output power and a 45-second exposure time for three subsequent exposures to test the antibacterial effects of 940 nm diode lasers on E. faecalis bacteria. The outcomes supported the findings of the current investigation, showing a decrease in bacterial colonies between the time they were exposed to 940 nm diode laser light and before exposure (P value 0.001). (Hendi et al., 2021) E. faecalis was destroyed by a 940-nm diode laser in a study by Castelo et al. They observed a 70% rate of bacterial killing by utilizing 3.5 watts of laser power in pulsed mode over a one-minute exposure length. More bacterial colonies were destroyed in the current study than in Castelo et al.'s examination because laser radiation of the bacterial suspension was applied continuously for 30 seconds, and this was consistent with the findings of the current study since 1 watt of power has the greatest ability to kill bacteria. (CASTELO et al., 2012)

One of the most popular disinfecting methods in dentistry for avoiding and inhibiting the growth of bacteria, particularly S. mutans, is chemical disinfection with chlorhexidine. (Mohan et al., 2016) Standard 2% CHX was employed in this investigation as a positive control because it is a disinfectant that is widely accessible on the market. The results of the investigation demonstrated that 2% CHX had high antibacterial properties and was effective against the S. mutans bacterium, as demonstrated by a decrease in CFUs/ml that provided a statistically significant difference (P value 0.001) when compared to the negative control group .

The results of this study agree with those of Vinothkumar, T. S. et al. They found that treating with 2% CHX greatly decreased the number of S. mutans and that diode laser was somewhat more effective at killing S. mutans than CHX. In comparison, CHX displays lesser antimicrobial properties than the diode laser, and this might be for various reasons, one of which is the photothermal effect of the laser. (Vinothkumar et al., 2020) The disinfectant's effectiveness is based on how well CHX sticks to microbe cell walls and lets intracellular components leak out. Small molecular weight components of the microorganism are released by CHX's bacteriostatic activity at low concentrations, but at higher concentrations, CHX causes cytoplasmic precipitation and/or coagulation, which is most likely brought on by protein cross-linkage and exhibits the bactericidal effect. (Mohan et al., 2016) According to the Hassaballah et al. investigation, the diode laser system is a more effective disinfectant for caries lesions than CHX and grape seed extract (Hasaballah et al., 2021). The study examined the efficacy of grape seed extract, CHX, and laser diode as primary disinfectants for dental cavities. There is agreement with the results of the present study, given that the outcomes of the diode laser group are better than those of the CHX group with a static difference (P value 0.001). When a laser beam contacts a tissue, a phenomenon known as photothermal interaction takes place in which the light energy is transformed into heat through a thermal interaction with the cellular molecules. It causes tissue to experience a number of effects, including heat, coagulation, vaporization, carbonization, and lastly, melting (Coluzzi, 2008). Theoretically, the intense absorption of laser light might cause the production of reactive oxygen species, which would kill bacteria directly by rupturing their cell membranes. (Yao et al., 2012)

5. Conclusion

According to the outcomes achieved from this study, the 940 nm diode laser has an antibacterial effect against bacteria that can cause tooth decay. So, it can be used as a potent disinfectant as a step in the tooth restoration process to help in bacterial elimination and cavity disinfection

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دراسة مختبرية للخاصية المضادة للبكتيريا لليزر الصمام الثنائي 940 نانومتر ضد بكتيريا المكورات العقدية الطافرة المعزولة من تسوس الأسنان

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الخلاصة

الخلفية: العنصر المسبب الرئيسي لتسوس الأسنان هو بكتيريا المكورات العقدية الطافرة، لذا فإن التخلص من هذه البكتيريا له تأثير كبير على مدى جودة العلاج الترميمي. تم تطوير أساليب جديدة للقضاء على البكتيريا في طب الأسنان مثل الليزر والجسيمات النانوية المعدنية والمواد النشطة بيولوجيًا. الهدف من الدراسة: كان هدف هذه الدراسة هو تقييم فعالية ليزر الصمام الثنائي كعامل مضاد للبكتيريا ثم مقارنته بالتأثير المضاد للبكتيريا للكورهيكسيدين ضد البكتيريا.

المادة والطريقة: أجريت الدراسة على المكورات العقدية الطافرة بتركيز 10^6. تم الحصول على عينات من البكتريا من تسوس الأسنان ثم تم تحضير المعلق البكتيري ووضعه في أنبوب إيبندورف ليتم معالجته بمختلف الأشكال المضادة للبكتيريا، وقسمت العينات إلى ثلاث مجموعات تجريبية:المجموعة أ: مجموعة التحكم السلبية؛ المجموعة ب: مجموعة المراقبة الإيجابية باستخدام 2٪ كلورهيكسيدين ؛ المجموعة ج: التشعيع بليزرالصمام الثنائي (قدرة 1 واط لمدة 30 ثانية). تم حساب عدد الوحدات المكونة للمستعمرات لكل مجموعة بعد 24 ساعة من الحضانة على اطباق الوسيط المتخصص للبكتيريا.

النتائج: لوحظ انخفاض كبير في اعداد البكتيريا. أظهرت النتائج فرقًا إحصائيًا) قيمة ($p < 0.01$) لكلا المجموعتين مقارنة بمجموعة التحكم السالبة بدون علاج ، وأعطت مجموعة ليزر الصمام الثنائي أكبر انخفاض في اعداد البكتيريا من مجموعة الكلوروهكسيدين.

الاستنتاج: كان ليزر الصمام الثنائي وسيلة ناجحة وفعالة في القضاء على البكتيريا ، ويمكن استخدامه كخطوة في عملية ترميم الأسنان .