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EVALUATION OF PHOTODYNAMIC THERAPY IN MICROORGANISMS ASSOCIATED WITH ORAL INFECTIONS: IN VITRO STUDY

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ABSTRACT: This study aimed to investigate the effectiveness of Photodynamic Therapy (PDT) in vitro against oral microorganisms, with emphasis on Streptococcus viridans. These microorganisms are associated with oral bacteremia and infectious endocarditis, highlighting the importance of exploring alternative treatments in light of the challenges of antibiotic therapy, such as side effects and microbial resistance. A quantitative experimental approach was conducted, testing different energy levels on S. viridans colonies. A red diode laser (660 nm) was used in combination with methylene blue 0.01% as the photosensitizer. The applied energy levels were 9 J (90s), 10 J (100s), 13 J (130s), 16 J (160s), and 18 J (180s). Samples were cultured in appropriate media and exposed to PDT under controlled conditions, following standardized protocols to assess post-treatment microbial viability. Lower energy levels (9 J and 10 J) had limited effects. It is concluded that PDT is a promising alternative for the control of S. viridans, particularly at higher energy doses, with potential as an adjuvant therapy for oral infections, reducing antibiotic dependence and microbial resistance risks. Further studies are required to optimize parameters and evaluate in vivo efficacy.

Keywords: Photodynamic Therapy. Streptococcus viridans. Diode laser. Oral infections.

RESUMO: Este estudo teve como objetivo investigar a eficácia da terapia fotodinâmica (TFD) in vitro sobre microrganismos presentes na cavidade oral, com ênfase em Streptococcus viridans. Esses microrganismos estão associados a bacteremias de origem oral e endocardite bacteriana infecciosa, tornando-se relevante a investigação de tratamentos alternativos, considerando os desafios atuais da antibioticoterapia, como efeitos colaterais e resistência microbiana. Por meio de uma abordagem experimental quantitativa, foram testados diferentes níveis de energia em amostras de colônias de Streptococcus viridans. Foi utilizando um laser de diodo vermelho com comprimento de onda de 660 nm, o fotossensibilizante de escolha foi o azul de metileno na concentração de 0,01% e os níveis de energia aplicados foram 9 J (90s), 10 J (100s), 13 J (130s), 16 J (160s) e 18J (180s). As amostras foram cultivadas em meio adequado e expostas à TFD em condições controladas, seguindo protocolos padronizados para avaliação da viabilidade microbiana pós-tratamento. Os resultados demonstraram que a terapia fotodinâmica foi eficaz na redução das colônias de Streptococcus viridans, com variação na efetividade conforme a dose de energia aplicada. As energias de 16 J, 18 J apresentaram os melhores resultados, com redução significativa ($p < 0,05$) na viabilidade bacteriana, enquanto doses menores (9 J e 10 J) mostraram efeito limitado. A técnica apresenta potencial para aplicação clínica como adjuvante no tratamento de infecções orais associadas a esses microrganismos, reduzindo a dependência de antibióticos e minimizando o risco de resistência microbiana. Estudos futuros são necessários para otimizar parâmetros e avaliar a eficácia in vivo.

Palavras-chave: TFD. PDT. LLLT. In vitro. Streptococcus Viridans.

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1. INTRODUCTION

The use of light (solar radiation or artificial light) as a form of therapy in medicine is mentioned in texts predating the 19th century found in Ancient Egypt and Ancient Greece. However, the first rigorous scientific documentation on light therapy in modern medicine was published by Niels Finsen (1893)(5) in the article "Om Lysets Indvirkninger paa Huden." His pioneering work not only consolidated the foundations of phototherapy, but also propelled the study of red light in the medical and scientific community. Currently, Photodynamic Therapy (PDT) is widely used in medicine, especially in dentistry. PDT consists of the combination of red light (emitted by a low-power diode laser) and a photosensitizer, resulting in the elimination of pathogenic microorganisms thru processes such as apoptosis, necrosis, and oxidative damage (TARDIVO, J. P. et al, 2005)(17).

Photodynamic Therapy (PDT) was introduced in Dentistry in the 1990s, with pioneering research focused on microbial control in oral infections (Wilson et al., 1992)(16). In the 2000s, the publication of the book Photodynamic Antimicrobial Chemotherapy (PACT) (WAINWRIGHT, 1998) represented a significant advancement in the dental literature on the subject. From 2010 onward, PDT has consolidated itself as an adjunctive therapy in Endodontics (Meire et al., 2012)(9) and Implantology (DORTBUDAK, 2015)(2), standing out as a promising alternative in the face of the challenges of antibiotic therapy, such as side effects and the growing problem of microbial resistance.

The present study aimed to evaluate the in vitro effects of a diode laser with a wavelength of 660 nm on Streptococcus of the viridans group (S. viridans), applied at varying energy levels. The research was conducted in the state of Espírito Santo, at Anhanguera College in Linhares, with the support of the Espírito Santo Research and Innovation Support Foundation (FAPES) and the Scientific Initiation program of COGNA Educação. Factors such as the energy level and the concentration of the photosensitizer are current research topics, as a gold standard protocol for the application of Photodynamic Therapy (PDT) in the elimination of pathogenic microorganisms has not yet been established. Streptococcus viridans are associated with oral infections and bacteremias (Wilson et al., 2021)(17). Particularly, S. sanguinis and S. mitis stand out as common etiological agents of infective endocarditis, due to their ability to adhere to damaged heart valves (WANG et al., 2020)(15). This bacterial group is characterized by being composed of Gram-positive cocci, catalase-negative, and α-hemolytic, in addition to producing dextran (S. mutans) and levan (S.

salivarius). The choice of these microorganisms is justified by their clinical relevance, as they make up the human microbiota, primarily colonizing the oral cavity, upper respiratory tract, gastrointestinal tract, and genitourinary tract, in addition to actively participating in biofilm formation (HABIB et al., 2021)(7).

This study investigated the efficacy of Photodynamic Therapy (PDT) on bacterial strains of Streptococcus viridans, using in vitro models to assess bacterial viability and susceptibility with an emphasis on the complete eradication of bacterial colonies. The results may contribute to the development of more effective and safer clinical protocols for the control of oral infections, offering a promising alternative to conventional antimicrobial therapies (TARDIVO et al., 2005; FIMPLE et al., 2008)(13,4).

2. Literature Review

2.1 Photodynamic therapy

In antimicrobial photodynamic therapy, the laser acts as a light source to activate the photosensitizer, promoting photochemical reactions in the tissues. The light emitted by the laser, usually red, is absorbed by the photosensitizer, which enters an excited state and generates reactive oxygen species. These species cause oxidative damage to the cellular structures of microorganisms, such as membranes and DNA, leading to their inactivation. The choice of laser wavelength is of great importance, as it influences the depth of penetration into tissues and the efficiency of photosensitizer activation, with longer wavelengths favoring greater tissue penetration (CIEPLIK et al., 2018)(1).

2.2 Photosensitizer: Methylene Blue

According to Marinho (2006), methylene blue is a photosensitizer widely used in photodynamic therapy due to its ability to generate reactive oxygen species (ROS) when activated by light at wavelengths of 660 nm. When irradiated, it transitions to an excited state and transfers energy to the molecular oxygen present in the medium, forming species such as singlet oxygen, which are highly reactive and capable of inducing cellular damage (MARINHO, S. A, 2006)(8).

2.3 Laser therapy in dentistry

Low-intensity laser therapy has established itself as an effective and safe therapeutic tool in dental practice, being widely used by dental professionals to complement treatments, mainly in the red (632-660 nm) and infrared (820-940 nm) ranges. While red radiation acts more superficially on tissues, infrared has a greater penetration capacity, reaching deeper tissues (NETO et al., 2020)(12).

Its analgesic, anti-inflammatory, and biostimulant effects, thru the increase in ATP production, fibroblast proliferation, and consequent collagen production, significantly contribute to pain reduction and the acceleration of the healing process. Moreover, due to its lack of significant side effects, laser therapy stands out as a safe non-pharmacological alternative, provided it is applied by properly trained professionals, respecting the specific technical parameters of each clinical case (NETO et al., 2020).

2.4 Viridans Group Streptococci

Viridans group streptococci are part of a set of commensal bacteria that mainly inhabit the oral cavity and the upper respiratory tract, being recognized for their ability to form biofilms, which are organized structures of bacterial cells that adhere to surfaces and are enveloped by an extracellular matrix. This ability is especially relevant in environments such as dental surfaces and medical devices, where biofilms provide protection against antimicrobial agents and favor the persistence of infection (SILVA et al., 2022)(15).

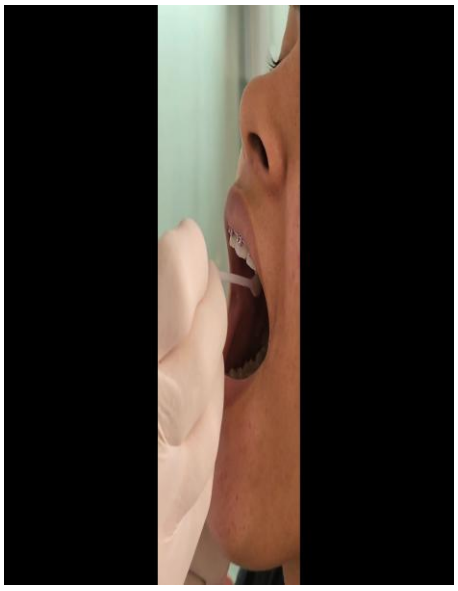
According to Oliveira (2024)(10), Streptococcus of the viridans group is one of the main microorganisms involved in the etiology of bacterial endocarditis of oral origin, being part of the oral commensal microbiota but capable of becoming pathogenic in situations of bacteremia. During invasive dental procedures or even in everyday activities, such as chewing or tooth brushing, these microorganisms can enter the bloodstream, especially in individuals with pre-existing heart conditions. It is estimated that S. viridans is responsible for about 30% of cases of bacterial endocarditis, reinforcing the importance of prevention and control of oral infections as a measure of systemic protection

3 Methodology

3.1 Selection of the bacterial strain

To obtain the Streptococcus strain of the viridans group, samples of the oral mucosa were collected using a sterile swab (THEDA et al., 2018)(14). Sampling was performed on the buccal mucosa, with axial rotation of the swab during the procedure. The samples were immediately inoculated onto blood agar plates with qualitative streaking and incubated at 37 °C for 24 hours. After bacterial growth, colonies with an alpha-hemolysis profile (partial hemolysis), a typical characteristic of the viridans group, were selected, and the GRAM staining test was performed to select gram-positive cocci, followed by the catalase test to differentiate Streptococcus from Staphylococcus (FERNANDES, G. et al, 2021)(3). For species confirmation, the colonies were subjected to the Optochin susceptibility test, with only those resistant being selected, which allows for the differentiation of Streptococcus from the viridans group, which exhibits intrinsic resistance to Optochin, unlike Streptococcus pneumoniae, common in the oropharynx and nasopharynx, which shows sensitivity to the Optochin test (SANTIN, K. et al.)(11).

Figure 1 - Collection of biological material with a sterile swab



Source: Authorship

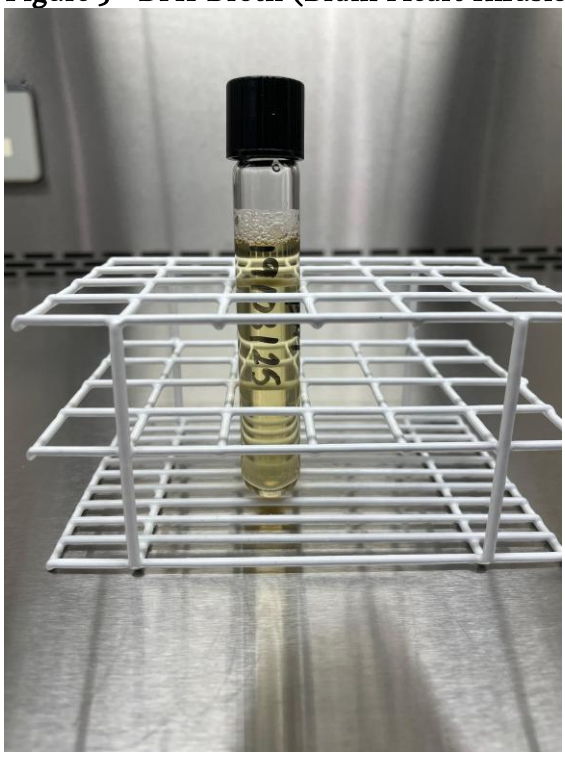
Figure 2 - Alpha-hemolytic strains isolated on blood agar

Source: Authorship

3.2 Preparation for Irradiation

The selected colonies were transferred to tubes containing BHI Broth (Brain Heart Infusion), an enrichment medium suitable for the growth of fastidious bacteria (FREIRE, I. C. M. et al, 2014)(6). Each tube received two to three colonies, and the samples were incubated for 24 hours at 37°C. This time was standardized based on preliminary observations, which indicated that after 24 hours of growth in BHI broth, the bacteria showed no viability when re-cultured on blood agar, which could compromise the colony count after treatment. The standardization time for incubation (24 hours) was determined based on preliminary observations, which showed that after this period in BHI broth, the bacteria in the control group showed no growth when re-cultured on blood agar, confirming the maximum time before loss of viability for CFU count accuracy. This observation was possible due to the lack of bacterial growth in the control group.

Figure 3 - BHI Broth (Brain Heart Infusion)



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3.3 Irradiation

After incubation, a 1:1 dilution of BHI and sterile saline was performed, with the aim of reducing the bacterial inoculum to facilitate colony counting after irradiation. After dilution, a 10 μL aliquot of the BHI Broth was taken for the tests. The samples were exposed to the methylene blue photosensitizer for 5 minutes, at a concentration of 0.01%, followed by irradiation with a low-power red laser, with a wavelength of 660 nm (MARINHO S, 2006)(8). The energies used were 9 J, 10 J, 13 J, 16 J, and 18 J with the Laser Duo (MM Optics). The power of 100 mW was used to determine the irradiation time, as energy (J) is the product of power (W) and time (s), where E = P x t. Thus, for the highest energy of 18 J, the time was 18J/ 0.1W = 180s. For each energy condition, a control group was included, which did not receive the dye and was not irradiated with laser.

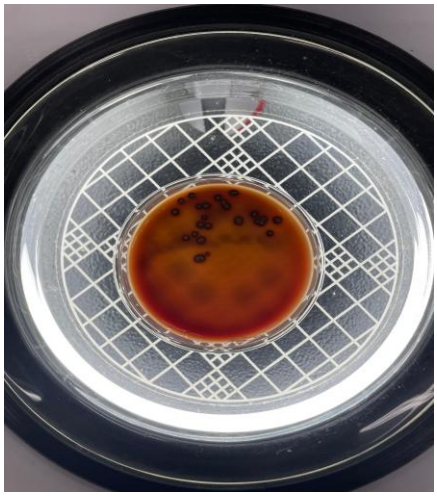
Figure 4 – MMOptics Laser Duo

Source: Authorship

3.4 Post-Irradiation Procedure

After the treatment, each irradiated aliquot was re-plated on blood agar plates using a 1μL bacteriological loop and incubated for 48 hours at 37°C (FREIRE, I. C. M. et al, 2014)(6). The colony-forming unit (CFU) count was performed to evaluate the antimicrobial effect of photodynamic therapy.

Figure 4 – CFU count after irradiation



Source: Authorship

3.5 Experimental Control

All tests were conducted with a control group, maintained under the same experimental conditions, except for the absence of methylene blue and laser irradiation in the control group. In addition, the same laser device was used in all the tests conducted. This approach allowed for a direct comparison between the tested and non-tested groups, ensuring the reliability of the obtained results.

4 RESULTS

The effectiveness of Photodynamic Therapy (PDT) was evaluated thru the counting of Colony Forming Units (CFU) (MARINHO, S. A, 2006)(8) after exposure of the bacterial samples to 0.01% methylene blue dye and irradiation with low-power laser at a wavelength of 660 nm. The applied energies were 9 J, 10 J, 13 J, 16 J, and 18 J. The results were compared with the control group, which did not receive dye or irradiation.

Table 1 - CFU Count per Experiment

Energy(J)	Average CFU	Percentage reduction compared to control
Control	>100.000UFC	—
9 J	40.000 ± 10^3	60%
10 J	35.000 ± 10^3	65%
13 J	29.000 ± 10^3	71%
16 J	50.000 ± 10^3	50%
18 J	26.000 ± 10^3	76%

Source: Authorship

The comparative analysis was conducted using the CFU count after 48 hours of plate incubation. A significant percentage difference was observed between all irradiated groups and the control group, with the highest antimicrobial efficacy observed atenergy of 18 J.

5 DISCUSSION

The results obtained demonstrate that Photodynamic Therapy (PDT), using 0.01% methylene blue dye associated with low-power 660nm red laser irradiation, shows significant efficacy in reducing Colony Forming Units (CFUs) of bacteria when compared to the control group.

A general dose-dependent response trend was observed, where the increase in applied energy resulted in a greater reduction of CFU, except for the 16 J energy, which showed lower efficacy, possibly due to variations in the inoculum or the post-irradiation plating procedure, but it ensured a decrease in colony count compared to the control group. As presented in Table 1, the energy of 18 J showed the highest antimicrobial efficacy, with a 76% reduction compared to the control.

It is important to highlight that the count in the control group exceeded 100,000 CFU, being considered the maximum reference for comparative purposes, due to the analytical limitation of the manual colony counting method. Even so, the data reinforces the potential of TFD as a promising approach for antimicrobial therapy.

Thru the experiments conducted, it was not possible to achieve the eradication of bacterial species cultivated in vitro thru TFD. Although the results demonstrated a significant reduction in the CFU count, the goal of completely eliminating the bacteria thru TFD was not achieved.

6 CONCLUSION

The results obtained in the present study show significant efficacy in reducing colony-forming units (CFUs) thru the application of photodynamic therapy (PDT). Although it was not possible to achieve complete eradication of the bacteria, the study reinforces the potential of PDT as a promising approach in the treatment of infections caused by resistant microorganisms.

Thru the research conducted, the benefits of TFD in bacterial reduction became explicit; however, the observed results were not sufficient to determine a protocol for the

clinical in vivo application of TFD. In the current academic and scientific context, it is necessary for new in vitro studies to be conducted, contributing to the development of in vivo therapies, aiming at the creation of clinical protocols with scientific evidence.

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