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COMPARATIVE EVALUATION OF ANTIBACTERIAL EFFICACY OF DIFFERENT ROOT CANAL IRRIGANTS WHEN ACTIVATED WITH DIODE LASER IN CANALS CONTAMINATED WITH ENTEROCOCCUS FAECALIS: AN IN VITRO STUDY

Endodontics

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ABSTRACT

Aim: The aim of this in-vitro study was to evaluate the antibacterial efficacy of different root canal irrigants namely normal saline, 3% NaOCl, 2% chlorhexidine, 0.2% chitosan with activation by diode laser in elimination of E. faecalis. **Materials And Methods:** A total of forty extracted single rooted teeth were decoronated and canal patency was established by #15-K file followed by biomechanical preparation. Enterococcus faecalis (ATCC 29212) was cultured and inoculated inside these root canals followed by incubation at 37C for 48 hours and divided into five groups (n=8) namely Group 1- irrigation with normal saline along with diode laser activation, Group 2- irrigation with 3% NaOCl along with diode laser activation, Group 3- irrigation with 2% chlorhexidine along with diode laser activation, Group 4- irrigation with 0.2% chitosan along with diode laser activation, Group 5- diode laser activation alone and the samples from the canal were collected with sterile paper points and cultured to evaluate the number of colonies formed after 24 hours of irrigation. **Results:** The mean of number of colonies formed after 24 hours were significantly less in Group 3 higher number of colonies were noted in group 1.**Conclusion:** From this study, it was concluded that 2% chlorhexidine along with diode laser activation showed the highest elimination of E. faecalis from the root canals.

KEYWORDS

Chlorhexidine, Chitosan, Diode laser, Disinfection, E. faecalis, Sodium hypochlorite

INTRODUCTION:

In endodontic treatment, thorough debridement of root canals is essential. However, due to the intricate structure of root canal systems, it is not possible to create a sterile area in infected root canals using mechanical preparation alone. Almost half of the root canal walls remain unprepared whether using conventional stainless-steel hand tools or the modern nickel-titanium instrumentation devices [1]. The primary contributing factor to the failure of endodontic treatment is microorganisms. The mere absence of bacteria in the root canal system for endodontic treatment to be successful. Even with careful mechanical preparation, residual pulpal tissue, germs, and dentine debris may remain in the abnormalities of root canal. Therefore, irrigant solutions should be used in combination with canal preparation [2].

Because of its numerous virulence factor genes, Enterococcus faecalis is the most difficult bacteria to eradicate in cases of persistent endodontic infections, where it is frequently encountered. Zoletti et al. discovered that 80% of 50 teeth that had received root canal therapy were infected with E. faecalis in an in vitro test [3]. Furthermore, Wang et al. found that E. faecalis infections were more common in teeth with poor obturation, having been found in 38% of the 58 teeth they analysed that had mediocre root canal operations [4].

In order to remove debris from inside the instrumented root canals, dissolve organic tissue remnants, sanitize the root canal area, and lubricate the instrumentation process without irritating biological tissues, an irrigant is used [5].

Sodium hypochlorite (NaOCl) has been a common root canal irrigant because of its strong antibacterial properties and capacity to dissolve tissue. However, it has a number of unfavourable qualities, including tissue toxicity, the possibility of developing subcutaneous emphysema, the possibility of allergies, and an off-putting taste and odour [6].

Because of its strong antibacterial activity and substantivity, chlorhexidine (CHX) digluconate has been recommended as a root canal irrigant. It appears to work by adhering to microorganisms' cell walls, which causes intracellular components to flow out.

It is bacteriostatic at low concentrations. has bactericidal effects at greater concentrations because it causes intracellular components to precipitate and coagulate [6].

Because of its natural polysaccharide properties—biocompatibility, biodegradability, bioadhesion, and lack of toxicity—chitosan has garnered interest in dental research. Chitin, which is abundant in nature and inexpensive to produce, is converted into chitosan by deacetylation. Chitin is found in the shells of crabs and shrimp (Kurita 1998) [7] . A variety of applications have made it an ecological interest. This 2% chitosan gel containing 0.1% chlorhexidine has been proven to have an antifungal action against Candida albicans in dentistry, and when it is added to calcium hydroxide paste as an intracanal medicine, it has been shown to prolong calcium ion release[6].

Over the past 15 years, a lot of research has been done on the application of lasers in endodontic therapy. It has been demonstrated that laser treatments have numerous benefits over traditional techniques. The findings imply that the laser is a useful instrument for disinfection as well as for clearing away trash, smear layers, and obturation materials. The second-best laser source is an 810 nm diode laser after Nd:YAG lasers. According to microbiological research, this source offers the second-highest reduction of microorganisms, at roughly 63% [8].

Combining the effect of root canal irrigants along with diode laser irradiation might potentiate the treatment outcome in endodontics. Hence, the aim of this invitro study is to evaluate the antibacterial efficacy of root canal irrigants with activation by diode laser in elimination of E. faecalis.

MATERIALS AND METHODS:

Sample preparation: Forty freshly extracted human mandibular

premolars were collected, decoronated using diamond disk and mandrel and standardised to a length of 14mm. The canal patency was established using 15 K file followed by biomechanical preparation using Protaper Gold rotary system up to size F3 using normal saline as irrigant.

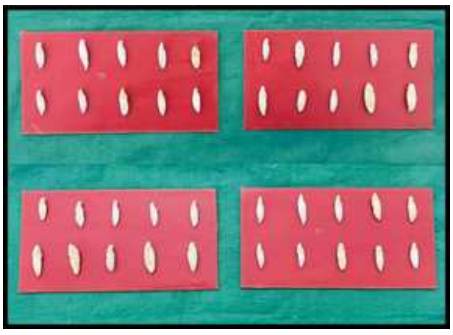


Fig 1 : Extracted Human Mandibular Premolars

The roots were sterilized in an autoclave for 15 min at 121°C and 15lb of pressure to ensure complete sterilization within the canal space. A suspension of E. faecalis (ATCC 29212) were adjusted to 0.5 turbidity on the McFarland scale (1.5×10^8 bacteria/mL). E. faecalis suspension of 10 µL was injected into the sterilised root samples. Then inoculated specimens with E. faecalis were placed in vials filled with BHI agar, then inoculum was added every day and incubated aerobically at 37°C for 3 days.

Fig 2 : E.faecalis Inoculation In The Root Samples

E. faecalis has ability to form biofilm on root canal walls. After 3 days, aliquots were taken from each tooth using a syringe and plated on BHI agar to verify the growth of E. faecalis.

Fig 3 : Laser Irradiation

At the end of 3 days, teeth were removed from the vials and excess fluid in canal was removed with sterile paper points. The specimens from each group were divided into five groups of 8 teeth each depending on the experimental irrigating solution used as Group 1 (n=8)- irrigation with normal saline along with diode laser activation, Group 2 (n=8)- irrigation with 3% NaOCl along with diode laser activation, Group 3 (n=8)- irrigation with 2% chlorhexidine along with diode laser activation, Group 4 (n=8)- irrigation with 0.2% chitosan along with diode laser activation, Group 5 (n=8)- diode laser activation alone.

The tip was placed 1mm short of working length and activated in a pulsed mode of 2 seconds and the probe was withdrawn in spiral motion at a rate of 2mm/second until complete canal irradiation was done followed by final flush with 5mL of distilled water to terminate the action of irrigant. Sterile absorbent paper point was used to obtain the sample from the canal. Culture of this sample was done on BHI agar. The plates were incubated at 37°C for 24 h and then number of colony forming units (CFUs) was counted.

RESULTS:

The Values obtained were statistically analyzed using Statistical Package for Social Sciences (SPSS) version 25.0. Post hoc tukey test was performed to identify the significant group. Mean and standard deviation were tabulated (Table 1). The result showed the least value in the group treated with 2% chlorhexidine activated by a diode laser, showing the best antibacterial effectiveness, having a bacterial count equal to 5.436×10^6 CFU/ml. This was followed by the 0.2% chitosan group, which amounted to 2.402×10^7 CFU/ml, the 3% NaOCl group with 7.567×10^8 CFU/ml, diode laser alone with 3.515×10^8 CFU/ml, and saline with the highest bacterial count of 1.284×10^9 CFU/ml.

Table-1: Mean comparison of CFU's between groups

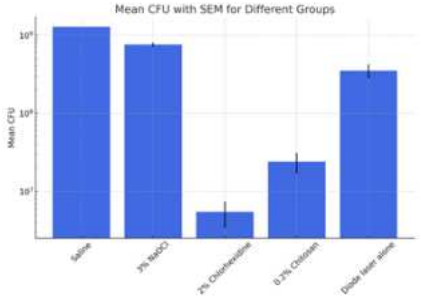
Group	Mean CFU	SEM
Saline	1.28×10^9	0
3% NaOCl	7.57×10^8	4.88×10^7
2% Chlorhexidine	5.44×10^6	2.03×10^6
0.2% Chitosan	2.40×10^7	7.11×10^6
Diode laser alone	3.51×10^8	7.00×10^7

The p-values indicate which pairs of groups have significantly different means. The results show significant differences in most comparisons except between 3% NaOCl vs. Diode laser alone, 2% Chlorhexidine vs. 0.2% Chitosan, and 0.2% Chitosan vs. Diode laser alone.

Table-2: Post hoc tukey test,p-value ≤ 0.05indicates statistical significance showing difference

Comparison	P value	Significance
Saline vs. 3% NaOCl	0.001	Significant
Saline vs. 2% Chlorhexidine	0.001	Significant
Saline vs. 0.2% Chitosan	0.001	Significant
Saline vs. Diode laser alone	0.001	Significant
3% NaOCl vs. 2% Chlorhexidine	0.001	Significant
3% NaOCl vs. 0.2% Chitosan	0.001	Significant
3% NaOCl vs. Diode laser alone	0.057	Not Significant
2% Chlorhexidine vs. 0.2% Chitosan	0.951	Not Significant
2% Chlorhexidine vs. Diode laser alone	0.034	Significant
0.2% Chitosan vs. Diode laser alone	0.193	Not Significant

Here is the bar graph representing the mean CFU values for different groups along with their respective Standard Errors of the Mean (SEM). The y-axis is plotted on a logarithmic scale to better visualize the differences between the groups. The error bars indicate the SEM for each group



DISCUSSION:

The purpose of this study was to evaluate the antibacterial effects of different root canal irrigants along with diode laser in elimination of E. faecalis from root canals. This particular bacteria was selected due to its remarkable resistance to various forms of endodontic therapy and frequent presence in recurrent endodontic cases. One of the challenges in getting rid of E. faecalis is that it can grow a biofilm inside the canal and biofilm bacteria are more challenging to eliminate than their planktonic counterparts.

This study used 3% NaOCl and 2% chlorhexidine as testing irrigants as they are most commonly used and considered the golden standard for

canal space sterilisation. Chitosan being an ecological material and its wide range of application in the medical field has been used in this study. As known, chitosan-acetate solution was found to cause significant removal of smear layer with minimal dentin erosion and eradication of biofilm might help to remove the E. faecalis from the root canals.

Diode laser being the most common laser found in clinical practice has been used as it use in endodontics and elimination of E. faecalis were documented in earlier studies.

The results of this study were in accordance with Benedicenti et al. (2008) [9], Schulte-Lünzum et al., [10] and Moritz et al., diode lasers were found to be effective in eliminating E. faecalis bacteria from biofilm. Although the laser's method of action against E. faecalis is not specifically described in any literature, Moritz et al. reported a response between the laser's ions and molecules on the cell wall. The bacterial cell membrane was eventually disrupted as a result of this process, which also damaged the protein molecules in the cell wall [11]. The bacteria undergo a significant metamorphosis at even the slightest disturbance of their membrane. Furthermore, it is thought that the laser beam's thermal action causes the cell membrane to rupture by raising its temperature by 42–52°C above its typical 37°C [12].

2% chlorhexidine along with diode laser irradiation showed better results in our study which is in consistent with the findings of Hmud et al., the creation and implosion of water vapor can cause cavitation on a water-based media when a diode laser with a wavelength of 980 nm is used.

The breakdown of the irrigation solution can be accelerated by bubble pressure from cavitation, improving disinfection. Bubble pressure in the root canal has the ability to break up the biofilms of microorganisms, tear cell membranes, and remove debris and the smear layer [13]. Although the response between diode lasers and CHX 2% has not yet been documented in any literature, Moritz (2006) has contended that the laser beam may alter the cellular molecule and structure. The laser beam's radiation changes and destroys the bacteria's cell wall, acting as a bactericide. This happens as a result of CHX using its biguanide content's positive ions to interact with the bacteria's cell wall's protein molecules' negative ions, causing lysis and osmotic disruption [11].

Higher degrees of deacetylation and lower molecular weight chitosan have been linked to increased antibacterial action, according to previous research. A higher level of chitosan deacetylation promotes the release of free amino groups, which interact with bacterial membranes to boost antibacterial activity [14]. Furthermore, it has been proposed that low molecular weight chitosan may bind to bacterial membranes more successfully because of its mobility, attraction, and ionic interactions with the negatively charged bacterial cell membranes [11].

In terms of irrigants, next to 2% chlorhexidine and 0.2% chitosan, 3% NaOCl showed the least number of colony forming units (CFU's) and this is consistent with Neelakantan et al. (2015), who concluded that the use of a diode laser improved results for a NaOCl 2.5% irrigant [15]. In addition, it was found that the combination of E. faecalis with a diode laser created a combined effect that maximized its antibacterial property.

This is due to the diode laser's thermal effect, which increases the capacity of the NaOCl 2.5% to eradicate E. faecalis, as well as the combination antibacterial action of the diode laser and NaOCl 2.5% [2].

CONCLUSION:

Diode lasers had an antibacterial effect on E. faecalis and this effect was enhanced when used along with potent irrigants like 2% chlorhexidine, 0.2% chitosan.

A more comprehensive understanding of the antibacterial effects of diode lasers in vivo will require more advanced research that simulates the clinical environment of the canal space using the required protocol based on clinical appearance. This preliminary study used direct contact between E. faecalis bacteria and the tested materials. However, a larger sample size is necessary to support the findings of this study.

REFERENCES:

1. Suzuki S. The Study of Chitosan-Citrate Solution as a Root Canal Irrigant: A Preliminary Report. Journal of Oral Hygiene & Health. 2014;02(04).
2. Sandini A, Meidyawati R, Kamizar, Npa DA. The antibacterial effect of a diode laser used as an adjunct irrigant on clinical isolate of Enterococcus faecalis biofilm (in vitro). Int J Appl Pharm.
3. Zoletti GO, Carmo FL, Pereira EM, Rosado AS, Siqueira JF Jr, Santos KR. Comparison of endodontic bacterial community structures in root-canal-treated teeth with or without apical periodontitis. J Med Microbiol 2010;59:1360-4.
4. Wang, Q.-Q., Zhang, C.-F., Chu, C.-H., & Zhu, X.-F. (2012). Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. International Journal of Oral Science, 4(1), 19–23.
5. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis: NaOCl and chlorhexidine. Int Endod J 2001;34(6):424–8.
6. Chandrasekhar V, Rao V, Rao A, Tummala M, Morisetty P, Elakanti S. Comparative evaluation of antimicrobial efficacy of QMix™2 in 1, sodium hypochlorite, and chlorhexidine against Enterococcus faecalis and Candida albicans. J Conserv Dent 2015;18(2):128.
7. Silva PV, Guedes DFC, Nakadi FV, Pécora JD, Cruz-Filho AM. Chitosan: a new solution for removal of smear layer after root canal instrumentation. Int Endod J 2013;46(4):332–8.
8. Sedgley CM, Lennan SL, Appelbe OK. Survival of Enterococcus faecalis in root canals ex vivo. Int Endod J 2005;38(10):735–42.
9. Benedicenti, S., Cassanelli, C., Signore, A., Ravera, G., & Angiero, F. (2008). Decontamination of Root Canals with the Gallium-Aluminum-Arsenide Laser: Anin VitroStudy. Photomedicine and Laser Surgery, 26(4), 367–370.
10. Schulte-Lünzum R, Gutknecht N, Conrads G, Franzen R. The impact of a 940nm diode laser with radial firing tip and bare end fiber tip on Enterococcus faecalis in the root canal wall dentin of bovine teeth: An in vitro study. Photomed Laser Surg 2017;35:357-63.
11. Moritz A, Schoop U. Lasers in endodontics. In: Oral Laser Application. Berlin: Quintessenz Verlags-Gmbh; 2006.
12. Bago I, Plecko V, Gabric Panduric D, Schauperl Z, Baraba A, Anic I. Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment. Int Endod J 2013;46:339-47.
13. Hmud R, Kahler WA, George R, Walsh LJ. Cavitational effects in aqueous endodontic irrigants generated by near-infrared lasers. J Endod 2010;36:275-8.
14. Supotngarmkul A, Panichuttra A, Ratisoontorn C, Nawachinda M, Matangkasombut O. Antibacterial property of chitosan against E. faecalis standard strain and clinical isolates. Dent Mater J 2020;39(3):456–63.
15. Neelakantan P, Cheng CQ, Ravichandran V, Mao T, Sriraman P, Sridharan S, Subbarao C, Sharma S, Kishen A, Photoactivation of curcumin and sodium hypochlorite to enhance antibiofilm efficacy in root canal dentin, Photodiagnosis and Photodynamic Therapy (2014)