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In Vitro Antimicrobial Activity Of Sodium Hypochlorite, Chlorhexidine And Ethylenediamine Tetra Acetic Acid As Irrigants Used In Endodontics

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Abstract

Introduction: The primary objectives of dental root canal therapy are to remove damaged tissue, eradicate the microorganisms in the dental root canal structure and avoid pulp space from being contaminated during surgery. The success rate of the procedure remains a concern, despite improvements in the instruments and procedures used in endodontic treatment. The major causes of failure and reinfection in endodontic procedures are colonisation of different forms of biofilm bacteria, development of a smear coating during instrumentation, intricate root-canal system morphology, and residual microorganisms in dentinal tubules. Although the mechanical instrumentation seeks to debridge contaminated channel walls, leakage from untouched root channel system areas cannot be eliminated. Because of these constraints, multiple irrigants are introduced to enhance the mechanical degradation process and supplement it.

Aim: The aim of the study was to evaluate the antimicrobial activity of Sodium hypochlorite, Chlorhexidine and EDTA irrigants used in endodontics against oral pathogens.

Materials and Method: The study deals with the broth dilution method that helps to determine the antimicrobial activity of irrigants by measuring the amount of colony forming units. The efficacy of the irrigants was evaluated by comparing with the control.

Result: Against Streptococcus mutans, among the 3 irrigants used, Sodium hypochlorite shows highest antimicrobial activity. Against Enterococcus faecalis, among the 3 irrigants used, Chlorhexidine shows highest antimicrobial activity.

Conclusion: The results show that there is no full elimination of bacteria. This could be a reason for the problems during root canal therapy. Further research is to be done on this topic for more accurate reasons for the failure of irrigants during root canal therapy.

Keywords: Irrigants, endodontics, bacteria, elimination, green energy, green synthesis, innovative technique.

Introduction

The primary objectives of dental root canal therapy are to remove damaged tissue, eradicate the microorganisms in the dental root canal structure and avoid pulp space from being contaminated during surgery (1). The success rate of the procedure remains a concern, despite improvements in the instruments and procedures used in endodontic treatment (2). The major causes of failure and reinfection in endodontic procedures are colonisation of different forms of biofilm bacteria, development of a smear coating during instrumentation, intricate root-canal system morphology, and residual microorganisms in dentinal tubules (3). Although the mechanical instrumentation seeks to debridge contaminated channel walls, leakage from untouched root channel system areas cannot be eliminated. Because of these constraints, multiple irrigants and medicaments are introduced to enhance the mechanical degradation process and supplement it (4). The use of many antimicrobial agents as irrigants and medicaments will make this possible. These substances are used mostly for relatively short periods of time (for irrigants) up to days or weeks (for medicaments) and hence their long-term antimicrobial activities are based on the fact whether or not the specific agent contains any substantive properties. If a successful residual long-lasting antimicrobial action of irrigants and medicaments could deter root canal system contamination, the long-lasting result of endodontic treatment could be increased.

Sodium hypochlorite (NaOCl) has been chosen as the gold standard to compare the antimicrobial effectiveness of irrigants (5). NaOCl is now the most commonly used irrigant and is almost the most powerful irrigant of many irrigation solutions used in endodontic therapies (6). NaOCl is used in various levels and higher levels are more potent in microbial elimination (7). NaOCl ionises in water as Sodium (Na^+) and hypochlorite ions (OCl^-) and strikes the hypochlorous equilibrium (HOCl). Most chlorine occurs as HOCl in acidic and neutral pH, while OCl^- is most prevalent with a pH of nine or above. The best antibacterial action is hydrochlorous acid, while the OCl^- ion is less effective. Hypochloric acid specifically impacts the microbial cell's basic functions which easily contributes to cell death (8).

EDTA, is a chelator used as the final irrigation agent after NaOCl (9). A neutral or very alkaline solution of EDTA; precipitates at acidic pH of EDTA. EDTA is typically a 17% or 15% solution, although some studies have shown that 5% or even 1% EDTA is sufficiently strong to remove the smear layer (10). The recommended time for removing string layers is around two minutes, but exposure times can be longer for thick layers (11). EDTA attacks only the inorganic portion of the smear and dentin layer (hydroxyapatite) and can only be fully removed if NaOCl has been employed with EDTA before the final rinse (12). EDTA however weakens the membrane of the bacterial cell without destroying the cell, but acts with other chemicals such as chlorhexidine, which destroys the cell wall more aggressively.

Due to its good antimicrobial activity, dentists use Chlorhexidine (CHX) for plaque prevention and disinfection (13). Also used as a final irrigant after EDTA in endodontics. CHX is cytotoxic to human cells, however may not induce pain, if extruded through the periapical region unintentionally (14). CHX does not dissolve organic or inorganic substances and cannot thus be used as the only solution for irrigation. CHX attacks the cell wall or external membrane of a microbial cell that kills the microbe (15). But planktonic bacteria are much slower to kill than NaOCl.

The pathogens that are chosen to test the antimicrobial activity of irrigants are Enterococcus faecalis and Streptococcus mutans. Enterococcus faecalis was chosen as one of the most commonly isolated species in persisting root canal infections as a facultative Gram positive anaerobic cocci (16). The ability of this bacterium to tolerate tough conditions with limited food and to stay viable for a long time in treated root canals was another justification for selecting E.faecalis. It also has the ability to thrive under extremely high conditions, acidity and hot conditions. Streptococcus mutans has also been chosen because it is a widespread microorganism in primary endodontic infections usually observed in failure to treat (17). It is the most predominant organism that is present in the oral cavity.

Our team has extensive knowledge and research experience that has translated into high quality publications(18–29), (30–34).

The aim of the study was to evaluate the antimicrobial activity of Sodium hypochlorite, Chlorhexidine and EDTA irrigants used in endodontics against oral pathogens.

MATERIALS AND METHOD

The Study was conducted in the Microbiology Laboratory, Saveetha Dental College, Chennai, India. The study deals with the broth dilution method that helps to determine the antimicrobial activity of irrigants used in endodontics by measuring the amount of colony forming units. Irrigants such as Sodium hypochlorite, Chlorhexidine and Ethylene diamine tetra acetic acid (EDTA) are taken in cuvettes. Then, 10µl is added to the bacterial suspension.The antimicrobial activity is tested against oral pathogens such as Streptococcus mutans and Enterococcus faecalis. Expose for 10 mins. Then, 10µl is taken in a pipette and transferred to a nutrient agar plate and uniformly spread. Then, incubated for 12 hrs and the total colony units are observed and tabulated. The efficacy of the irrigants was evaluated by comparing with the control.

RESULTS

Table 1: Table shows the total and mean value of the colony forming unit of Sodium hypochlorite.

Sodium hypochlorite	Sample 1	Sample 2	Sample 3	Total	Mean
Streptococcus mutans	36	30	42	108	36
Enterococcus faecalis	821	488	323	1632	544

Table 2: Table shows the total and mean value of the colony forming unit of Chlorhexidine.

Chlorhexidine	Sample 1	Sample 2	Sample 3	Total	Mean
Streptococcus mutans	60	62	136	258	86
Enterococcus faecalis	59	59	84	202	67.3

Table 3: Table shows the total and mean value of the colony forming unit of Ethylene diamine tetra acetic acid.

Ethylene diamine tetra acetic acid	Sample 1	Sample 2	Sample 3	Total	Mean
Streptococcus mutans	86	116	280	482	160.6
Enterococcus faecalis	702	627	347	1676	558.6

Table 4: Table showing the mean value of antimicrobial activity of Sodium hypochlorite, Chlorhexidine and Ethylene diamine tetra acetic acid.

	Sodium hypochlorite	Chlorhexidine	Ethylene diamine tetra acetic acid
Streptococcus mutans	36	86	160.6
Enterococcus faecalis	544	67.3	558.6

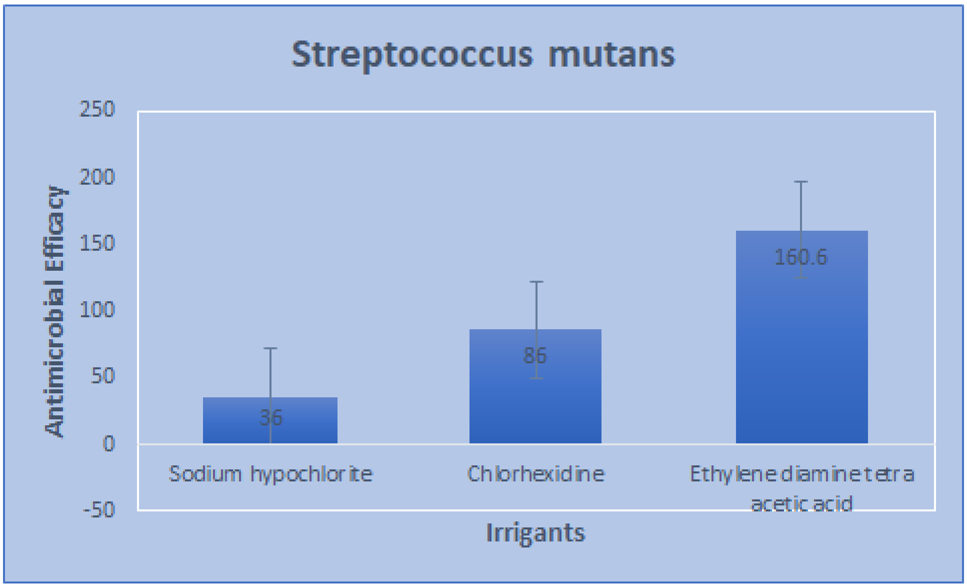

Figure 1: Bar chart represents the antimicrobial efficacy of Irrigants such as Sodium hypochlorite, Chlorhexidine and Ethylene diamine tetra acetic acid (EDTA) against Streptococcus mutans.

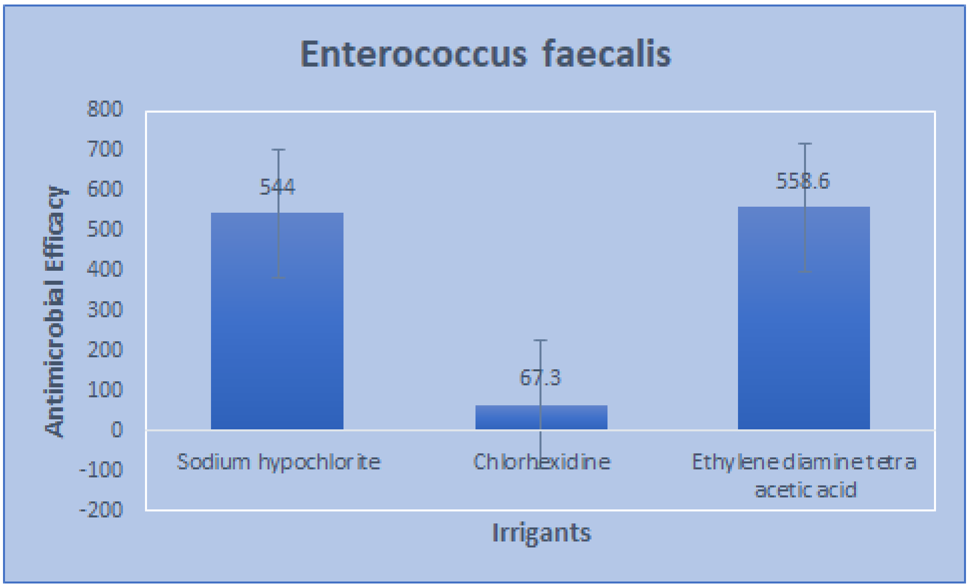

Figure 2: Bar chart represents the antimicrobial efficacy of Irrigants such as Sodium hypochlorite, Chlorhexidine and Ethylene diamine tetra acetic acid (EDTA) against Enterococcus faecalis

DISCUSSION

According to this study, the antimicrobial efficacy of Streptococcus mutans showed a mean value of total colony forming unit of 36 for Sodium hypochlorite, 86 for Chlorhexidine and 160.6 for EDTA. And the antimicrobial efficacy of Enterococcus faecalis showed a mean value of total colony forming unit of 544 for Sodium hypochlorite, 67.3 for Chlorhexidine and 558.6 for EDTA. One of the main goals of root canal therapy is root canal disinfection. The antimicrobial activity of Sodium hypochlorite, Chlorhexidine and EDTA irrigants used in endodontics against pathogens such as Streptococcus mutans and Enterococcus faecalis is summarised in table 1, 2 and 3 respectively. Table 4 shows the mean value of antimicrobial activity of Sodium hypochlorite, Chlorhexidine and Ethylene diamine tetra acetic acid. The results show that there is no full elimination of bacteria. Figure 1 and 2 shows the bar chart representing the antimicrobial efficacy of Streptococcus mutans and Enterococcus faecalis. The above mentioned tables and charts show the total colony forming unit in the nutrient agar culture plates. The results showed that among the 3 irrigants used, the decreasing order of effective irrigants that can be used against Streptococcus mutans is observed in the order of Sodium hypochlorite > Chlorhexidine > EDTA. Among the 3 irrigants used, the decreasing order of effective irrigants that can be used against Enterococcus faecalis is observed in the order of Chlorhexidine > Sodium hypochlorite > EDTA. EDTA has little or no antimicrobial activity. This could be a reason for the problems during root canal therapy.

Sodium hypochlorite (NaOCl) has been chosen as the gold standard to compare the antimicrobial effectiveness of irrigants (35). NaOCl is now the most commonly used irrigant used in endodontic therapies (36). NaOCl is used in various levels and higher levels are more potent in microbial elimination. Berber et al. has shown that, in three different concentrations 0.5%, 2.5% and 5.25%, 5.25% of concentration of NaOCl is the most effective (37). It is clear that the increased accumulation of apical and periapical tissue has more deleterious effects. EDTA is a chelator, used as the final irrigation agent after NaOCl. EDTA is typically a 17% or 15% solution, although some studies have shown that 5% or even 1% EDTA is sufficiently strong to remove the smear layer (38). EDTA has little or no antimicrobial activity. EDTA however weakens the membrane of the bacterial cell without destroying the cell. Due to its good antimicrobial activity, dentists use Chlorhexidine (CHX) for plaque prevention and disinfection (39). Also used as a final irrigant after EDTA in endodontics. CHX attacks the cell wall or external membrane of a microbial cell that kills the microbe (40).

Different antimicrobial pathways are used in the available irrigant solutions, such as sodium hypochlorite (NaOCl), chlorhexidine (CHX), EDTA, etc. There are, however, a number of drawbacks in connection with each form (41). NaOCl has adequate antimicrobial properties as a typical solution of irrigants. However, it has an unpleasant taste which can cause edoema, necrosis of tissue, etc when used incorrectly. It is also detrimental to toxicity, possible to cause ill effects and is unable to dissolve the smear coat.

The Limitations of this study is that it is confined to a limited number of culture plates, irrigants and organisms. This study is conducted with only 3 irrigants placed in 9 culture plates and 2 culture plates as control.

The future scope of this study is that it can be done on a large number of culture plates, with more irrigant solutions and by testing it against various other organisms to know the accurate reason for failure of disinfecting the root canals.

CONCLUSION

Against Streptococcus mutans, among the 3 irrigants used, Sodium hypochlorite shows highest antimicrobial activity. Against Enterococcus faecalis, among the 3 irrigants used, Chlorhexidine shows the highest antimicrobial activity. The results show that there is no full elimination of bacteria. This could be a reason for the problems during root canal therapy. Further research is to be done on this topic for more accurate reasons for the failure of irrigants during root canal therapy.

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CONFLICT OF INTEREST

All the authors declare no conflict of interest in the study.

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