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Evaluation and Comparison of Antimicrobial Efficacy of New Irrigant Chitosan Citrate on Enterococcus Faecalis and its Potency to Remove Smear Layer: An Invitro Study

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Abstract: ***Aim: To evaluate and compare the antimicrobial efficacy of new irrigant Chitosan citrate on Enterococcus Feacalis and its potency to remove smear layer. Methods: The study was conducted in two parts. Part one is the assessment of antibacterial efficacy of five irrigants against E. faecalis. E. faecalis (29212) was incubated in brain-heart infusion broth. Sodium hypochlorite, chlorhexidine, EDTA, 10% citric acid (pH3.5), Chitosan-citrate solution was then added to the bacterial inoculum for 5, 15, 30, and 60 min. Survival rates of E. faecalis were analysed as number of colonies formed at each time interval. Part two is the assessment of efficacy of chitosan citrate and 10% citric acid in smear layer removal. Thirteen extracted teeth were used for this experiment. The middle third of the root was cut longitudinally, and all specimens were immersed in both solutions for five minutes. All specimens were examined under scanning electron microscopy. The number of open dentin tubules was determined for evaluation of smear layer removal. Mann Whitney and chi square tests were used for analysis of open dentin tubules. Results: The antibacterial effect of chitosan-citrate solution was achieved at 30 min and 60 minute. Chitosan-citrate solution removed significantly more of the smear layer than 10% citric acid (P<0.05). Conclusion: Chitosan-citrate solution showed antibacterial activity at 30 minutes and 60 minutes which shows it could be used as an intracanal medicament in primary tooth pulpectomy. Effectiveness of a chitosan-citrate solution to remove the smear layer in root canal treatment is satisfied and is superior to 10% citric acid . The chitosan-citrate solution might be the promising endodontic irrigation solution in future.***

Keywords: Enterococcus faecalis ; Chitosan citrate ; 10% citric acid ; Sodium hypochlorite; EDTA ; Chlorhexidine digluconate; Smear layer

1. Introduction

Successful endodontic treatment is based on cleaning and shaping and sealing of the root canal system.[1] It is impossible to create a sterile root canal space, only with mechanical preparation alone because of the complex anatomy of root canal systems.[2]Various studies have reported that root canal infection are polymicrobial in nature and most commonly predominated by obligate and facultative anaerobes.[3] So each step in root canal therapy aims to disinfect the root canal system and prevent reinfection in future. Hence achieving a complete sanitization becomes a rare reality, on the other aspect any necrotic tissue or microbial debries left behind can act as a nidus for microbial recolonization and could decrease the effects of root canal irrigants or medicaments and interfere with the adaptation of root canal fillings to dentin in the pulp space and thus resulting in failure of the treatment. Therefore, good irrigation system for thorough debridement becomes an essential part of root canal treatment as it allows for cleaning beyond the mechanical preparation.

Repeated endodontic failures occur mainly due to incomplete removal of pulp tissue or micro-organisms present within the root canals where they survive and are different from microbiota normally found in untreated teeth. Literature suggests that bacteria most frequently found in first time treatments, as well as treatments with infectious recurrence, are limited to a very specific variety of micro-organisms, where gram positive anaerobic facultative species predominate, especially Enterococcus faecalis which colonizes and infect dentin tubules complicating its removal through chemical and mechanical cleansing.[3]These micro-organisms might have survived biochemical procedures or have invaded root canals through crown ſiltration in teeth with ſilled roots. Several studies have revealed the fact that microbiota present in root canals of endodontically treated roots differ from microbiota normally found in untreated teeth. This bacteria has been isolated within root canal systems as well as in periapical lesions. It has equally been found in infected teeth that had not been previously endodontically treated.[4] Nevertheless, it is most frequently observed in teeth that have suffered recurrence, that is to say failure of previous endodontic treatment.

So in order to predictably eliminate as many bacteria as possible from the entire root canal system, different methods and strategies have been advocated such as newer instrumentation techniques, variable irrigation regimens, intracanal medicaments and chemicals that remove the smear layer which harness the bacteria like E.Feacalis. Studies endorse that a combination of mechanical instrumentation, chemical debridement and intracanal medication can be employed to achieve the goal of endodontic treatment. Various irrigants with the said properties have been cited in literature with favourable clinical success, among them sodium hypochlorite, chlorhexidine gluconate, EDTA have shown good results.

Sodium hypochlorite is the most widely recommended irrigating solution in endodontics with good clinical success due to its capacity to dissolve necrotic tissue remants, low cost and a very effective microbial activity against microbiota of infected root canals, however its cytotoxic,

mutagenic nature and its impact on dentin matrix and occasional complications or accidents have been well cited in the literature.[5]

Chlorhexidine has got a wide range of activity against both Gram positive / negative bacteria. Its cytotoxic effect on human fibroblasts[6] and osteoblasts indicate that it can impair the regenerative potential of the periapical tissues.[5]

The other possible cause for failure is the formation of smear layer due to cutting of dentin during cleaning and shaping.[8] Removal of smear layer is a highly controversial issue, as presence of smear layer itself may be infected and could harbor bacteria within the dentinal tubules.[9]This is significant in teeth with infected root canal system where the outcome of the endodontic treatment depends on the elimination of bacteria and their byproducts from the root canal system.[10]Traditionally, myriad of compounds in aqueous solutions have been suggested as root canal irrigants for removal of the smear layer including inert substances such as saline or acids like citric acid, lactic acid, tannic, polyacrylic acid or chelator solutions like bis-dequalinium acetate, EDTA. Chelating agents like citric acid and EDTA are highly biocompatible and safe to use but they have little or no antibacterial effect. Most of the solutions used have the disadvantages that it has to be used in combinations with other solution to have cent percent efficiency. So there is no one material which can satisfy all the ideal requirements of an ideal irrigant till date. Hence there is continuing quest to find a material which fulfils all the requirements and is also safe and free of adverse effects in children.

For fulfilling all these requirements, a newer innovative solution, a natural polysaccharide comprising of copolymers of glucosamine and N-acetylglucosamine called chitosan which is produced by partial deacetylation of chitin has gained attention in dental research because of its biocompatibility, biodegradability, bioadhesion and lack of toxicity. In addition it also possess a good antimicrobial activity.[8]In this study weevaluated and compared the antimicrobial efficacy of new irrigant chitosan citrate on E. feacalis and its potency to remove smear layer for the improved success of endodontic treatment in children.

2. Materials and Methodology

The study included 13 freshly extracted single-rooted human teeth. After extraction, all the teeth were placed in a 2.5% NaOCl solution for 15 min. The tissue and debris remnants on the root surface were then removed andwere stored in a 0.9% saline solution with thymol. The teeth were free of caries, cracks, root canal treatment and restorations.The teeth were free of caries, cracks, root canal treatment, internal resorption and restorations

It was done under two parts, first part of the study was aimed to access the antimicrobial assessment of five irrigants against E. Faecalis and data was collected based on the number of colony forming units. Second part of the study is the evaluation of efficacy in removing smear layer between two irrigants and the specimens were analyzed under scanning electron microscope.

Preparation of chitosan-citrate solution: The pH of 10% citric acid was adjusted with sodium hydroxide. 0.04 g chitosan oligosaccharide was added to 1 mL of 10% citrate buffer solution, and the mixture was stirred until completely dissolved. The pH of the chitosan citrate solution was adjusted at 3.5.

Assessment of antibacterial properties of five different solution: Enterococcus faecalis was incubated overnight at 37ºC in brain-heart infusion (BHI) broth. 10 µl of planktonic bacterial cells were transferred into each test tube. The tubes were centrifuged (10000 g x 5 min) and supernatants were discarded. Sodium hypochlorite (Group I),EDTA (Group II), chlorhexidine (Group III), 10% citric acid (Group IV) andChitosan-citrate solution (Group V) were tested. 100 µl of test solution were added to the bacterial inoculum for 5 minutes, 15 minutes, 30 minutes and 60 minutes and was incubated in blood agar plate for 48 hours at 37ºC. Colonies were counted after 48 hours of incubation at 37ºC, and expressed as colony-forming units (CFU) per milliliter. The antimicrobial effect of all Groups was recorded at 5 minutes, 15 minutes, 30 minutes and 60 minutes.

Assessment of smear layer removal: Thirteen extracted human permanent teeth were selected based on their relative dimensions and similarity in morphology. All teeth were stored in 0.1% thymol solution before use and was decoronated at the cemento-enamel junction with a water-cooled carborundum disk. The canals of 15 roots were instrumented according to the step back technique with a Gates-Glidden drills and K-type files to No.35 at the working length. Irrigation during instrumentation was performed with 1 mL 5.25% sodium hypochlorite after using each file. Upon completion of instrumentation, the apical and coronal thirds of each root were removed. The middle third of the root were cut longitudinally, and twenty six specimens were prepared. Thirteen specimens each were immersed in chitosan-citrate solution (pH3.5), 10% citric acid (pH3.5) for 5 minutes. After performing these procedures, all specimens were dehydrated in t-Butyl alcohol. Later they were mounted on aluminium stubs, vacuum dried and the middle third of root were examined under a scanning electron microscope. The number of opened, partially opened and closed dentin tubules seen in the field determined the degree of smear layer removal. The degree of evaluation was scored in a blind manner based on a three-grade scale by an examiner who was not informed about the true nature and purpose of this study. The root canal cleanliness was qualitatively assessed at the middle region of each root half of each specimen using a graded scale from 0 to 2 to assess the quality of smear layer removal according to Kanter et al. (0 – completely opened tubules with complete smear layer removal, 1– partially opened tubules, and 2 – no open tubules).

The collected data were analysed using Chi-Square and Mann-Whitney U tests. P values were computed and compared with statistical significance at the p=0.05 level.

3. Results

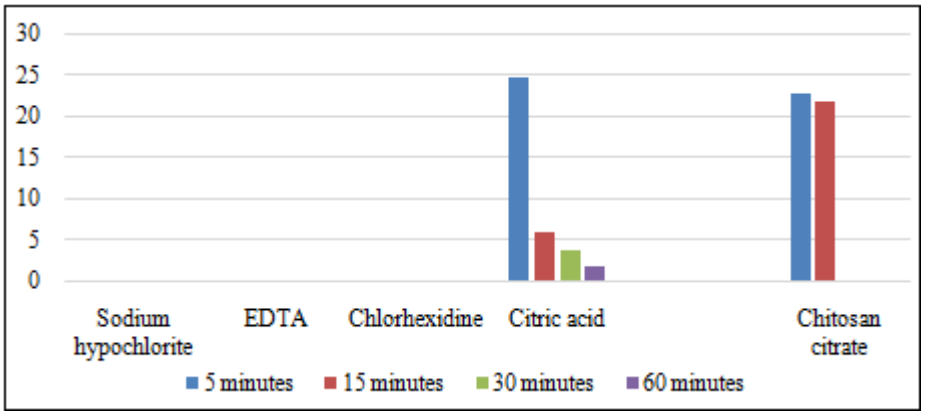
The antimicrobial assessment of five irrigants against E. Faecalis is presented in Table 1and Graph 1 showing the

number of colonies formed at 5, 15, 30 and 60 minutes in each solution.

	Colony forming units (Time in minutes)			
	5 minutes	15 minutes	30 minutes	60 minutes
Group I	0	0	0	0
Group II	0	0	0	0
Group III	0	0	0	0
Group IV	25	6	4	2
Group V	23	22	0	0

Table 1 showing the number of colonies formed at 5, 15, 30 and 60 minutes in each solution.

Table 1 and Graph 1shows there were no colonies seen at 5, 15, 30 and 60 minutes for Group I,II and group III denoting their high efficacy against E.faecalis. Group V showed minimal efficacy at 5 and 15 minutes but there was a drastic decrease in the number of colonies formed. All the colonies were disappeared at 30 and 60 minutes indicating its high efficiency at these time intervals.



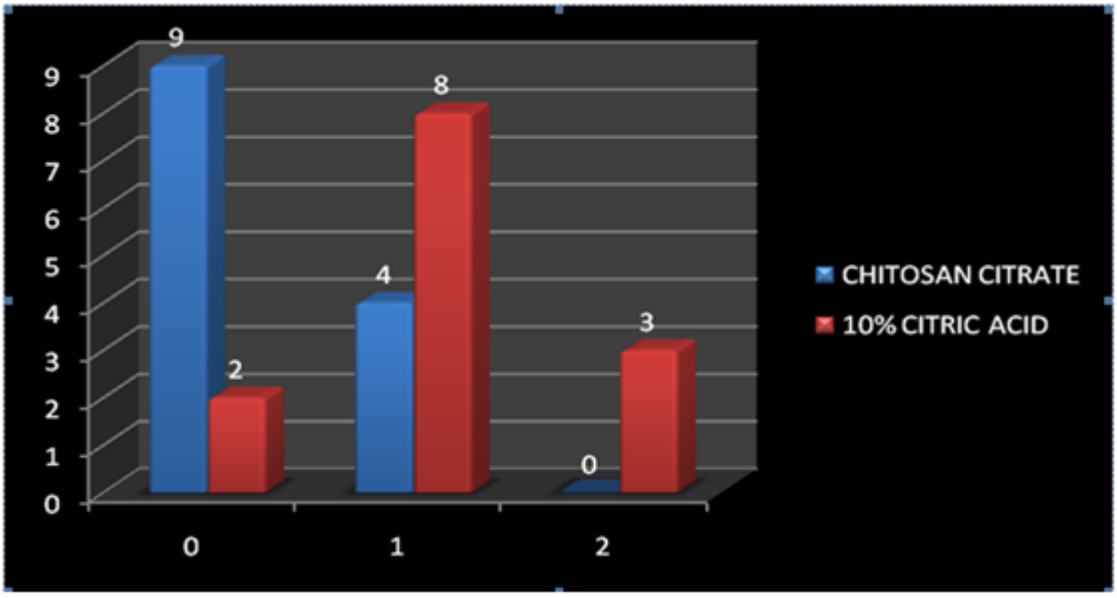
Graph 1: Shows the number of colonies formed at 5, 15, 30 and 60 minutes

Group IV shows minimal efficacy at 5 minutes with highest number of colonies, but there was reduction in the number at 15, 30 and 60 minutes treated. However, complete antibacterial efficacy was not achieved even at 60 minutes.

Assessment of efficacy of chitosan citrate and 10% citric acid in smear layer removal is shown in Table 2 and Graph 2

Score	Group- number of samples	
	Chitosan citrate (Group 1)	10% citric acid (Group2)
0	9	2
1	4	8
2	0	3

Table 2 shows the number of opened dentinal tubules in each group



Graph 2: shows the number of opened dentinal tubules in each group

Table2 and Graph 2 shows the number of open dentinal tubules in group 1 and group 2. In group 1 out of thirteen samples, nine (69.2%) of them showed complete opened dentinal tubules whereas in group 2 showed only two samples (15.4%). Four samples (30.8%) in group 1 showed partially opened tubules whereas in group 2 showed eight (61.5%). None of the samples in group 1 showed closed dentinal tubules but in group 2 showed three samples (23.1%) with closed dentinal tubules.

Table 3 denotes the efficacy of smear layer removal of chitosan citrate and 10% citric acid in percentage.

			GP		
			1 Chitosan Citrate	10% Citric Acid	Total
	0	Count	9	2	11
		% within CC	81.80%	18.20%	100.00%
		% within GP	69.20%	15.40%	42.30%
	1	Count	4	8	12
		% within CC	33.30%	66.70%	100.00%
		% within GP	30.80%	61.50%	46.20%
	2	Count	0	3	3
		% within CC	0.00%	100.00%	100.00%
		% within GP	0.00%	23.10%	11.50%
Total		Count	13	13	26
		% within CC	50.00%	50.00%	100.00%

Table 3 shows complete opening of the dentinal tubules were seen in 69.2% of specimens in group 1 whereas only 15.4% of the specimens in group 2 showed complete dentinal tubule opening which was statistically significant.

In 30.8% of the specimens in group 1 showed partial opening of the dentinal tubules whereas 61.5% of the specimens had partial opening in group 2 which was also statistically significant.

Thus there were no specimens in group 1 without smear layer removal. However in group 2 23.1% had completely closed dentinal tubules.

Table 4: Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.788[a]	2	.012

Table 5: Test Statistics[b]

	CC
Mann-Whitney U	33.000
Wilcoxon W	124.000
Z	-2.907
Asymp.Sig. (2 - tailed)	.004

Table 4 and table 5 shows the statistical analysis done for this study which was analysed using mannwhitney test and chi square test and the results were found to be statistically significant ($p<0.05$)

4. Discussion

The bacterial flora of the root canal has been studied for over many years and has long been recognized as the primary etiologic factors in the development of pulp and periapical lesions.[11,12] Endodontic treatment primarily focuses at debridement procedures to disrupt and remove the microbial ecosystem that is associated with the disease process. It is important that clinicians understand the close relationship between the presence of microorganisms and endodontic disease processes in order to develop an effective rationale for treatment.

Current concepts suggest that the number of bacterial species in an infected root canals may vary from one to more than 12 and the number of bacterial cells from <102 to >108 per sample.[13]Number of studies has shown that invasion of bacteria into dentin tubules occurs in 60-90% of teeth with apical periodontitis.[14]

A review **by Vytaute Peciuliene et al (2008)** reported that microbiological findings from filled root canals with persistent periapical disease have shown a high proportion of enterococci, ranging from 29% to 77%.[4]A study by **Gajan et al** foundthat E. faecalis was found to be the most prevalent species causing the secondary infection in root canal treated teeth.[3]According to **Evans M et al**E. faecalis has some virulence factors which could be the reason for survival of this microorganism in a very harsh environment of the root canal system which includes: secreted factors, adhesins, surface structures such as capsular polysaccharide and antibiotic resistance determinant. E. faecalis has special capacities as endopathogen: to invade dentinal tubules and adhere to dentin surface.[15] Number of studies showed another extremely important characteristic of this microorganism: capacity to withstand a wide pH range up to around 11.5 of intracanal medicaments such as calcium hydroxide which is generally a highly potent antimicrobial dressing.[15]In a study conducted by **Davies JK**the mechanism of alkaline tolerance of this microorganism was shown and it was associated with a functioning cell-wall-associated proton pump, which drives protons into the cell in order to acidify the cytoplasm. It was believed that E. faecalis is the microorganism which can withstand high pH of intracanal dressings like calcium hydroxide and play a critical role for its involvement in persistent infection in endodontic retreatment cases. Another characteristic of enterococci is an ability to survive even in the environment of low nutrient supply. This property was explored in a series of long-term starvation assays.[15]Therefore E. faecalis was chosen as test micro organism in the present study.

Chitosan is a natural polysaccharide comprising of copolymers of glucosamine and N-acetylglucosamine which is produced by partial deacetylation of chitin which is obtained from shells of crustaceans and shrimps. Studies conducted by**De-Deus G et al** and **Darrag AM et al** showed thatchitosan possesses high chelating capacity [16] and has properties of biocompatibility, bioadhesion, biodegradability, andantimicrobialactivity.[17]**Siqueira Jr JF et al** foundcitric acid alone could not provide a good antibacterial effect and good chelating effect simultaneously. Therefore, we considered chitosan as a material that supplements the capacity of citric acid. So far, the combination of chitosan and citric acid has been reported to be used as the liquid component of calcium phosphate cement for bone substitute materials.[18] According to **Varshosaz J et al,** citric acid was used as a cross linking agent to produce drug delivery chitosan film.[19]

In this study it was evaluated that the antibacterial activity against planktonic E. faecalis and removal of the smear layer when using a newer irrigant chitosan-citrate solution (pH 3.5)**.** The antibacterial activity of chitosan-citrate solution were depended on the application time i.e. 5 minutes, 15 minutes, 30 minutes and 60 minutes. Here, a complete bactericidal effect was not achieved at 5 and 15 minutes. It is similar to the study conducted by **Pedro FL et al 2018**, who showed that chitosanwere not effective in completely

eliminating dentin bacterial contamination with E. faecalis.[20]This findings were in accordance with **Witedja et al 2018**were they compared the effectiveness between 1–3% chitosan acetate (CA) and 1–3% chitosan citrate (CC) against E. faecalis biofilm formation after treatment for 15, 30, and 60 minutes. However, in their study they have found that chitosan acetate to have a better efficacy when compared to chitosan citrate, which was possibly due to lower solubility of chitosan in 10 %citric acid when compared to 1% acetic acid.[21]This can be attributed to the reduced efficacy of chitosan citrate at 5 and 15 minutes. But studies have showed that antimicrobial efficacy of chitosan citrate is solely based on the action of chitosan as the antibacterial effect due to the pH of chitosan citrate is weak.[1]In this study, an increased antibacterial efficacy was seen during 30 and 60 minutes which can be due to the time dependent dissolution of chitosan in 10% citric acid. Thus studies have to be conducted in the future with higher concentrations of citric acid and the solubility of chitosan at this elevated concentration.

But a significant antibacterial efficacy was however noted at 30 and 60 minutes interval.So this result shows that chitosan citrate in the root canal for a prolonged period can produce a significant antibacterial efficacy which could justify its use as an intra canal medicament. These findings are in accordance with the invitro study conducted by **Zhila Imani et al** to evaluate the Antibacterial Effects of Chitosan, Formocresol and CMCP as Pulpectomy Medicament on Enterococcus faecalis, which showed that there is an obvious antibacterial efficacy of chitosan as a medicament in pulpectomies of infectious primary teeth.[22]

According to **Sudarshan NR et al**the antimicrobial action of chitosan is said to be the presence of the positively charged NH3 + groups of glucosamine that interacts with negatively charged surface components of bacteria, resulting in extensive cell surface attraction, leakage of intracellular substances, and causing damage to vital bacterial activities.[23]In addition to it **Pankaj Yadavet al** foundchitosan binds to DNA and inhibits mRNA synthesis by penetrating toward the nuclei of microorganisms and interfering with the synthesis of mRNA and proteins.[24]

In our study we compared the removal of smear layer with chitosan citrate and 10% citric acid and we found chitosan-citrate solution removed significantly more of the smear layer than 10% citric acid when immersed for 5 min with a significant p value of 0.04. It is important to emphasize that the scores attributed to chitosan-citrate solution were higher than those given to 10% citric acid, that is, chitosan-citrate solution resulted in superior removal of smear layer than 10% citric acid. These findings are in accordance with **Shigenori Suzukiet al**[2] and **M. Praveen et al**[25]The formershowed that Chitosan-citrate solution removed significantly more smear layer than 10% citric acid ($P<0.05$)[2] and the latter showed that the use of Chitosan Citrate as final rinse solution during biomechanical preparation seems promising.[25]This superior smear layer removal capacity can be attributed to the properties of chitosan.

Researchers have hypothesized two theories on the mechanism involved in chelating with chitosan. The first theory is based on the bridge model, where the same metal ion is bound by two or more amino groups on the chitosan chain.[26] The second theory claims that only one of the amino groups on the chitosan chain is anchored to the metal ion.[27]Many dimers of chitin combine to form a chitosan polymer. The polymer is similar to that found in the EDTA molecule. The chitin dimer comprises of nitrogen atoms that combine with free electrons which are responsible for the ionic reaction with the metal and the chelating molecule. Based on a chemistry analysis of chitosan, the amino groups in the chitosan chain (in acidic medium) are known to have a positron charge based on bi-polymer protonation. The phenomenon is associated with the attraction of metal ion to the chelating agent.[28]Further analysis revealed that chitosan and metal ion complexes are a result of ion exchange, chelation, and adsorption.[28] The chelating interaction is dependent on the type of interaction, pH of the solution, and the structure of chitosan.[29]

In this study, citric acid was used for comparing with chitosan citrate for the removal of smear layer. **Goldman et al.** reported that the effects on the removal of the smear layer obtained with citric acid were similar to those by EDTA.[30]**Ando reported** that citric acid is less cytotoxically irritable to tissue than EDTA.[31]

Citric acid is a chelating agent that reacts with metals to form a nonionic soluble chelate**.** This study showsthat citric acid has the ability of removal of smear layer but was found to be inferior to chitosan citrate. These findings are in accordance with **Shigenori Suzuki et al** showed that Chitosan-citrate solution removed significantly more of the smear layer than 10% citric acid ($P<0.05$).[2]

Here, citric acid showed the least antibacterial efficacy against E.Feacalis, which was in accordance with the studies conducted by **Kaushik et al** and **Moliz et al,** however its primary function as endodontic irrigant is removing the smear layer and any antibacterial property can be considered as an additional advantage.[32] Thus they can be used in the removal of contaminated smear layer.

Present study confirms that sodium hypochlorite showed antibacterial efficacy against E. faecalis at 5 minutes,15 minutes,30 minutes and 60 minutes.These findings were in accordance with the study done by **Ercan et al.** to evaluatethe antibacterial activity of 2% Chlorhexidine as irrigating solution in infected teeth compared to5.25% NaOCl. Counting of CFU (Colony Forming Units) in samples obtained from the root canals before and after chemo-mechanical preparation showed that both Chlorhexidine gluconate and sodium hypoclorite were significantly effective to reduce the endodontic pathogens in teeth with periapical pathologies.[33]But it was in contrast with the study conducted by **Jeansonne e and White**comparing 2% Chlorhexidine and 5.25% NaOCl in vitro, showed that Sodium hypochlorite was not that effective in reducing the number of positive culture, even if the difference was not statistically significant.[34] The result of this study was similar to the **Rôças IN et al** whoreported in their study both 2.5% sodium hypochlorite (NaOCl) and

0.12% chlorhexidine digluconate (CHX) have antimicrobial acivity against E.Feacalis.[35] It is also in accordance with the study conducted by **Gomes BP et al** and **V. B. Berber et al**.[36] Former showed that both irrigants NaOCl and Chlorhexidine possessed antibacterial activity and the the time required to eliminate E. faecalis depended on the concentration and form of the irrigant used. Latter showed among of 0.5%, 2.5% and 5.25% concentration of sodium hypochlorite (NaOCl), at higher concentrations of NaOCl, showed the highest antibacterial effect against E, feacalis.[36]

NaOCl is formed by the hypochlorite anion (OCl^-) and hypochlorous acid (HOCl) in different proportions, which in combination promote the release of free chlorine[37]. This gives NaOCl protein-dissolving ability and antimicrobial activity. Furthermore, due to its high alkalinity, sodium hydroxide, resulting from the dissociation of organic material, has dissolving capacity through saponification of fatty acids.

According to **Senna RA et al**[38]the bactericidal ability of sodium hypochlorite results from the formation of Hypochlorous acid (HOCl) when in contact with organic debris. At lower pH, the chlorine in the NaOCl solution is predomi-nantly available as HOCl, more active than the OCl– that is prevalent at a more alkaline pH. . HOCl has a powerful bactericidal effect, since it is able to better penetrate the bacterial cell membrane due to its lack of electrical charge and its molecular structure. Hypochlorous acid exerts its effects by oxidizing sulfhydryl groups within the bacterial enzyme system, thereby disrupting the metabolism of the microorganisms resulting in killing of the bacterial cells.Once within the cell,HOCl has a bactericidal effect, reacting with DNA, RNA, and other nucleotides. Additionally, this substance acts as a bac-teriostatic agent, reacting with amino acids to produce chloramines.

In our study antimicrobial activity of 17% EDTA was obtained at 5 minutes, ieits antibacterial activity against E. faecalis is in par with the NaOCl and Chlorhexidneirrigants. EDTA has not shown an obvious antibacterial effect against E. faecalis in a study conducted by**Zhang R et al**.[39]Our results is in contrast with study conducted by **Rui Zhang** et al who conducted comparative study between EDTA, chlorhexidine, and some other irrigantson their antibacterial and antimicrobial activities against Enterococcus faecalis and it was concluded that chlorhexidine showed the strongest and longest activity.[39]

According to **Arias-Moliz et al.,** antibacterial activity of EDTA is said to be attributed to its chelating ability where it removes the inorganic component of the smear layer and changes the permeability of the cell membrane. The contrasting finding in other studies may be attributed to the increased surface tension and small permeability of EDTA which made it difficult to penetrate into the dentine tubules to kill the E. faecalis exhaustively.[40] But in this study we have only assessed the antimicrobial efficacy without taking into consideration about the permeability of the solution.

Results of this study confirmsthat chlorhexidine also showed antibacterial efficacy against E. faecalis at 5 minutes. These findings were in accordance with **Natasha Jaiswal et al (2017)** showed that, Chlorhexidine, Chitosan + Chlorhexidine and Propolis were found to be as efficacious as sodium hypochlorite.[41] These findings was also in similar with the study conducted by **Jeansonne e and White** comparing 2% Chlorhexidine and 5.25% NaOCl in vitro, showed that Chlorhexidine was more effective in reducing the number of positive culture, even if the difference was not statistically significant.[34]It is in contrast with **Rôças IN et al (2011)** reported in his study both 2.5% sodium hypochlorite (NaOCl) and 0.12% chlorhexidine digluconate (CHX) have antimicrobial acivity against E. Feacalis where NaOCl showed more than chlorhexine.[35] It was also in accordance with the study conducted by **Gomes BP et al**showed that both irrigants NaOCl and Chlorhexidine possessed antibacterial activity and the time required to eliminate E. faecalis depended on the concentration and form of the irrigant used.[36]

CHX is a cationic bis-biguanide with good efficacy against several gram-positive and gram-negative bacteria found in endodontic infections according to **Siqueira Jr JF**its antibacterial effects are likely to be related to the induction of damage to the bacterial cytoplasmic membrane and precipitation of intracellular constituents and its substantivity property (i.e., continued antimicrobial effect CHX binds to hard tissue and remains antimicrobial),. It permeates the microbial cell wall or outer membrane and attacks the bacterial cytoplasmic or inner membrane or the yeast plasma membrane. In high concentrations, CHX causes coagulation of intracellular components.[18]

This study shows that chitosan citrate has a good antibacterial efficacy at 30 and 60 minutes. Hence future studies has to be conducted to assess its possible use as intra canal medicament in primary tooth pulpectomy. Antibacterial efficacy of chitosan citrate at different concentration has to be analysed. This is is an invitro study comparing the antibacterial efficacy and smear layer removal of four routinely used irrigant solutions. Future invivo studies has to be conducted so as to incorporate chitosan citrate into routine endodontic armamentarium.

5. Conclusion

This invitro study concludes that:

1) Chitosan citrate has significant antimicrobial activity against E faecalis at 30 and 60 minutes.
2) However, Chitosan citrate is inferior to sodium hypochlorite, EDTA and chlorhexidine at 5 and 15 minutes. This shows that chitosan citrate can be an effective intra canal medicament as it achieves its antibacterial efficacy at longer duration of contact and it should be validated with future researches.
3) Chitosan citrate has the ability to remove smear layer as there were no specimens with completely closed dentinal tubules.
4) Chitosan citrate removes significantly more smear layer when compared to citric acid and hence can be effectively used as an irrigant for smear layer removal.

Thus within the limitation of the study, chitosan citrate can be used as an effective root canal irrigant as a medium for

smear layer removal. Further studies have to be conducted on its anti-microbial efficacy.

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