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In-Vitro Evaluation of Antimicrobial Efficacy of Triple Antibiotic Paste with Chitosan Against Enterococcus Faecalis

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ABSTRACT

Aim: To evaluate the antimicrobial efficacy of triple antibiotic paste (ciprofloxacin, metronidazole and cefaclor) with chitosan, calcium hydroxide and normal saline against Enterococcus faecalis.

Introduction: An infected root canal system is a unique niche for selective yet multiple species of microorganisms. There are broad spectrum species especially Gram-positive facultatives, which possess greater resistance to antimicrobial agents used during endodontic treatment. To prevent reinfection of a treated root canal, it is mandatory to disinfect the pulp space & dentinal tubules thoroughly with an endodontic irrigant or intra canal medicaments.

Methodology: E. faecalis grown at a concentration of 1.5 $\times 10^8$ CFU/ml is inoculated into 21 extracted human teeth with single root canal and incubated. These teeth are divided into 3 groups based on medicament used. Each experimental canal is placed with intracanal dressing TAP + Chitosan, calcium hydroxide and Normal Saline in group 1, 2, 3. Dentinal shavings are collected at 24 hours and 21 days interval following which post colony counts for microbial evaluation are calculated.

Results: All groups except normal saline showed negligible microbial counts after 24 hours. At 21 day interval, there was significant increase in microbial count from 24 hours to 21 days. TAP+ chitosan had maximum antimicrobial efficacy while least by Calcium hydroxide.

Conclusion: From the findings, it can be concluded Triple antibiotic paste with chitosan can be used as possible alternative for intracanal dressing over longer period of time.

Keywords: *Intracanal medicaments, Triple antibiotic paste, chitosan, calcium hydroxide, enterococcus faecalis*

INTRODUCTION

A single visit endodontic therapy is gaining popularity, so the need of the hour is anti-bacterial agent that has high effectiveness to remove and prevent any re-growth of microorganisms.[1] An infected root canal system is a unique niche for selective yet multiple species of microorganisms. There are broad spectrum species especially Gram-positive facultatives, which possess greater resistance to antimicrobial agents used during endodontic treatment than anaerobes. Therefore, the major cause of post-treatment disease after RCT is persistence of these microorganisms deep into dentinal tubules. Higher prevalence of Enterococci species was observed in studies with. persistent reinfection.[2]

Of the enterococci species, Enterococcus faecalis has shown higher prevalence ranging about 29-77% in the teeth with root canal failures and persistent diseases due to its resistant nature. The reason for resistance are –

- Ability to grow in high salt concentration
- Proliferation under wide temperature range
- Tolerance to broad pH range.[3,4]

Application of local antibiotics within the root canal system has been seen to be effective to combat the resistance of facultative anaerobes. Selection of antimicrobial agents are based on susceptibility and resistance patterns of E. faecalis. Penicillin and ampicillin are not used because exclusive strains of E. faecalis express the β.lactamase enzyme; instead, amoxicillin with clavulanic acid is used. Ciprofloxacin and Clindamycin are nowadays popularly used and are reported to be effective against facultative and anaerobic odontogenic infections.

The combination of irrigants and medicaments decreases the development of resistant bacterial strains and produces synergistic effect, allowing prolonged antimicrobial action and sustained release of medicaments. To increase the stability, and diffusibility, chitosan can be used as a drug carrier.[5]

This study was thus undertaken to evaluate the antimicrobial efficacy of different intracanal medicaments-triple antibiotic paste (ciprofloxacin, metronidazole and cefaclor) with chitosan, calcium hydroxide and normal saline against Enterococcus faecalis.

MATERIALS & METHODS

Twenty one caries-free extracted human permanent teeth were collected from the Department of Pedodontics and Preventive Dentistry, I.T.S. Dental College, Hospital and Research Centre, Greater Noida and from other private clinics and government institutions for this study. Protocols in cross-infection control as per Occupational Safety and Health Administration regulations in storing, surfacing, and utilization were taken into full consideration.

The samples collected were according to following criteria.

Inclusion criteria:

1. Caries free teeth
2. Orthodontic extraction
3. Serial extraction
4. Extraction due to periodontal conditions

Exclusion criteria

1. Grossly carious teeth
2. Teeth affected due to developmental anomalies affecting the root
3. Teeth fractured while extraction

METHODOLOGY

MEDICAMENTS

TABLE 1. LIST OF MEDICAMENTS USED IN STUDY ALONG WITH THEIR CONCENRATIONS AND MANUFACTURERS

S.NO	MEDICAMENTS	CONCENTRATIONS	MANUFACTURERS
1.	3 MIX with Chitosan 3 MIX- • Ciprofloxacin • Metronidazole • Cefaclor Chitosan	 500 mg 400 mg 500mg	 CIPLA LTD, Sikkim India J.B. CHEMICALS & Pharmaceuticals Ltd. Distaclor mfd. By A.Menarini India Pvt. Ltd By nano research elements, India
2.	Calcium hydroxide		Neelkanth Health Care
3.	Normal saline	0.9%	Shree KrishnaKeshav laboratories Ltd.

SAMPLE SELECTION AND PREPARATION

After extraction, twenty one teeth selected for the study were cleaned, made free of any debris or deposits and were stored in a normal saline solution at 37ºC until they were to be used. All the remnants and calculus were removed with an ultrasonic scaler prior to root canal preparation.

Each specimen was decoronated and standardised at particular root length of 13 mm from cemento-enamel junction. Root canal preparation was done, for irrigation normal saline and EDTA was used. After the biomechanical preparation, paper points were used to dry the canals.

STERILIZATION

Specimens were transferred into sterilization reels for autoclaving and sterility was checked using sterilization indicator tape, which changes its colour from beige to black.

CULTURING OF BACTERIA & INNOCULATION

Brain heart infusion broth prepared was used for culturing of bacteria. A standard suspension of E. faecalis (ATCC 29212) at a concentration of 1.5×10^8 CFU /ml was prepared. Test tubes were incubated under anaerobic conditions to allow the growth of E.faecalis for 72 hrs. After 72hrs, growth was checked by presence of turbidity in test tubes. E.faecalis suspension was dispensed into three test tubes containing each 5 ml under aseptic conditions to prevent any cross-contamination. Seven teeth samples were immersed per test tube containing 5 ml of E. faecalis suspension and were incubated for 24 hrs in candle jar at 37^0C. After 24 hrs the inoculated teeth were centrifuged at 3300 rpm to allow penetration of E. faecalis deep into dentinal tubules. The inoculated teeth were stored at 37^0 C and 100% humidity for 72 hrs to ensure growth of E. faecalis.

The teeth were randomly divided into following three groups comprising of seven Steeth each based on medicament used.

PREPARATION OF 3 MIX WITH CHITOSAN .

100µg/ml concentration of each drug was prepared and mixed with 0.2% of chitosan and paste like consistency of 3 MIX with Chitosan was achieved .

After incubation period, each experimental canal was treated with the medicament according to the particular group. The medicament treated samples were stored at 37^0 C and 100% humidity for 24 hrs and 21 days interval after which growth was evaluated using digital colonimeter.

EVALUATING GROWTH OF BACTERIA

For evaluating the growth of bacteria at different time intervals, dentinal shavings were collected and vortexed solution was used for plating over the nutrient agar. The media was then incubated at 37^0C for 24 hrs. Following incubation,growth of E.faecalis was evaluated using digital counter at 24 hour and 21 day interval.

STATISTICAL ANALYSIS

Intergroup comparison for antibacterial effect between different groups for Non Parametric data. was tested using Kruskal Wallis. When the differences between groups were statistically significant, Bonferroni test with Post Hoc comparisons, Mann-Whitney test was used, for parametric and nonparametric data, respectively to detect means that are significantly different from each other.

RESULT

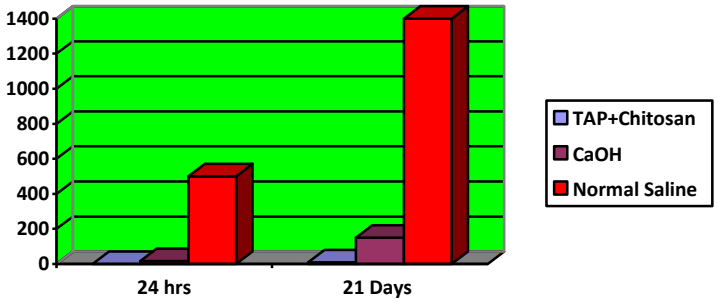
In the present study, a significant difference was found in the mean colony count at 24 hour interval where normal saline showed maximum microbial count while other groups showed negligble counts. It is well accepted fact normal saline has no antimcrobial activity. It only exerts its effect during mechanical prepartion of root canal.

(a) **(b)** **(c)**

Figure 1: 24- hour post medicament colonies of E. faecalis seen on Nutrient Agar with (a) TAP with chitosan (b) calcium hydroxide (c) Normal Saline

(a) **(b)** **(c)**

Figure 2: 21 days - post medicament colonies of E.faecalis seen on Nutrient Agar with (a) TAP with chitosan (b) calcium hydroxide(c) Normal Saline



GRAPH 1.

	Group 1 (TAP+Chitosan)	Group2 (CaOH)	Group 3 (NS)
At 24 Hours			
Mean	0.6	17	500.0
S.D.	0.8	9.1	47.5
Chi Sq. value- 21.5, p<0.001*			
AT 21 DAYS			
Mean	10	150	1400
S.D.	5.2	16.02	2597.2
Chi Sq. value- 23.7, p<0.001*			

Table 1 Graph and Table 1 - Mean Microbial colony counts after 24 hours and 21 days

Intergroup comparison (Table 1) using Kruskal Wallis Non Parametric Test reveals Significant relation in mean colony counts at 24 hours and 21 days interval among different groups

Bonferroni test with Post Hoc comparisons showed significant difference in mean colony count with TAP+ Chitosan with CaOH at hour, TAP+ chitosan also showed significant difference with NS at 24 hours, and 21 days interval

DISCUSSION

Root canal infections are polymicrobial in which anaerobic bacteria can make up as much as 90% of the cultivable flora. Polymicrobial infections are more pathogenic than those involving single organisms due to bacterial synergy (Sundqvist,1994).[6] Therefore, endodontic therapy aims to eliminate bacteria from the infected root canal and prevent infection.

The outcome of endodontic treatment is largely dependent on the effectiveness of control or elimination of microrganisms from the root canal system. This can be achieved with mechanical cleaning processes and through the use of chemical agents.[7]

Despite the use of many irrigants and intercanal medicaments like Calcium hydroxide[Ca(OH)2], certain microbial species do survive and causes reinfection. Enterococcus faecalis has long been

implicated as chief microbe for persistent root canal infections. It is the commonest species identified which is most commonly recovered from root canal with post treatment periapical infections[8]

Since root canal infections are poly microbial in nature and because of the complexity of infections, different combinations of antibiotics are needed to address the diverse microbial flora. Triple antibiotic paste with combination[9]. The antimicrobial efficacy of TAP alone and their combination was evaluated and studied by Hoshino et al. who demonstrated none of these antibiotics was able to completely remove the pathogens but the combination showed consistent sterilisation. Hoshino et al. recommended metronidazole (500 mg), minocycline (100mg) and ciprofloxacin (200mg) at 1:1:1 ratio for 3MIX formulation with carrier propylene glycol and macrogol ointment at 1:1 ratio [10]. This formulation was later modified by Takushige et al. as metronidazole, minocycline and ciprofloxacin mixed in a ratio of 3:3:1.[11]

In present study modified triple antibiotic paste (mTAP) along with carrier was used which included ciprofloxacin, metronidazole and cefaclor each in concentration of 100µg/dl to achieve the antimicrobial efficacy along with least cytotoxic effects. Our study thus compared the antimicrobial efficacy of mTAP with newer vehicle, i.e. chitosan, against E. faecalis with the aim to achieve maximum penetration within dentinal tubules.

The antimicrobial action of calcium hydroxide is mainly dependent upon direct contact with bacteria. It is not very effective in eliminating bacteria deep from the dentinal tubules. Enterococcus faecalis being small enough can proficiently invade and thrive within dentinal tubules. Orstavik et al reported in their that Enterococcus faecalis in the dentinal tubules are resistant to calcium hydroxide intracanal dressing over 10 days[12]

In the present study, a significant difference was found in the mean colony count at 24 hour interval where normal saline showed maximum microbial count while other groups showed ne gligble counts. It is well accepted fact normal saline has no antimcrobial activity. It only exerts its effect during mechanical prepartion of root canal.

At 24 hour interval ,our study showed similar results with Adl et al [13] where normal saline exhibted least antimicrobial activity and TAP showed maximum antimicrobial activity. According to their study, the TAP is more effective against E. faecalis compared to calcium hydroxide which is similar to our results.

At 21 day interval,TAP+ chitosan showed mean colony counts which was best among all the groups. Calcium hydoxide exhibited maximum colony counts after normal Saline which explains it having least antimicrobial effect when compared with TAP+Chitosan.

Pratik Kotadia et al. in their study demonstrated effectivness of using TAP + 2% chitosan which produced significantly better and faster wetting of the root canal walls as compared to $Ca(OH)_2$ + 2% chitosan[14]. Intra group comparison showed significant increase in microbial count from 24 hours to 21 days interval in CHX and $Ca(OH)_2$group which could be attributed to the reduced capability of $Ca(OH)_2$ in maintaing high pH.

Within the limitations of the study, it can be concluded that:

- At 24 all the groups showed antimicrobial efficacy.
- At 21 days interval triple antibiotic paste with chitosan demonstrated best antimicrobial efficacy.

CONCLUSION

Considering the antimicrobial efficacy and toxicity, Triple antibiotic paste with chitosan can be used as best possible alternative for intracanal dressing over longer period of time. However, further studies are warranted to establish the efficacy of intracanal medicaments against E. faecalis at longer time intervals.

Declaration by Authors

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