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Microbiological effect of different intracanal medicaments

Aiswarya Ajith¹, Rajan Dhawan^{1,*}, Shivani Dhawan², Jayata Dhawan³, Dikshant Tayal¹ & Ena Sharma⁴

¹Department of Conservative Dentistry and Endodontics, Maharishi Markandeshwar College of Dental Sciences and Research, Mullana, Ambala, Haryana, India; ²Department of Periodontology, Maharishi Markandeshwar College of Dental Sciences and Research, Mullana, Ambala, Haryana, India; ³Department of Pediatric and Preventive Dentistry, Maharishi Markandeshwar College of Dental Sciences and Research, Mullana, Ambala, Haryana, India; ⁴Corresponding author

Institutional URL:

E-mail: info@mmumullana.org

E-mail: info@rayatbahrauniversity.edu.in

Author contacts:

Aiswarya Ajith - E-mail – aiswaryaajith259@gmail.com; Phone - +91 8714411887
 Rajan Dhawan - E-mail – endorajan@mmumullana.org; Phone - +91 9541155555
 Shivani Dhawan - E-mail – shivani.dhawan@mmumullana.org; Phone - +91 7206310000
 Jayata Dhawan - E-mail – jayata.dhawan@mmumullana.org; Phone - +91 9068261143
 Tayal Dikshant - E-mail – dikshant9760@gmail.com; Phone - +91 9996889531
 Ena Sharma - E-mail – dr.sharma_ena@yahoo.co.in; Phone - +91 8284855768

Abstract:

The effect of Triple antibiotic paste (TAP), Modified triple antibiotic paste (MTAP) and its combination with Himalayan pink rock salt (HPRS) against *Enterococcus faecalis* is of interest to dentists. Intracanal medicaments were placed on specimens infected with EF and incubated. Antibacterial efficacy was evaluated by colony forming unit method on the 1st, 3rd, and 7th day. MTAP + HPRS showed the best results against *E. faecalis* on days 1 and 3 which were statistically significant. All subgroups showed zero colonies on day 7. HPRS combined with TAP/MTAP showed more antibacterial activity than TAP/MTP used without HPRS as intracanal medicament due to its synergistic effect. Hence, a modified triple antibiotic mixed with Himalayan pink rock salt can be used as a potential intracanal medicament with 2% Chlorhexidine.

Keywords: Intracanal medicament, *Enterococcus faecalis*, triple antibiotic paste, modified triple antibiotic paste, himalayan pink rock salt.

Background:

Endodontic treatment aims to eliminate microorganisms and necrotic pulp tissue from the root canal system, followed by shaping and enlarging the canal space to accept dense filling material, further sealed to create a barrier between periapical tissue and oral cavity [1]. The periapical tissues are invaded by microorganisms through the carious process, leading to pulp inflammation, necrosis, periapical periodontitis, and ultimately the formation of abscess [2]. In failed endodontic therapy, *Enterococcus faecalis* has been routinely identified. It is an anaerobic gram-positive coccus that typically commences in the human oral cavity, vagina, and gastrointestinal tract because it has confirmed excellent adaptation to such environments as low oxygen levels and rich in nutrients. The proton pump mechanism of bacteria causes protons to be transported into the cell to lower the cytoplasm's alkalinity and aid in its survival [3]. About 50% of the root canal system may remain un-instrumented despite chemo-mechanical preparation in complicated architecture like fins, deltas, and ramifications. After the canal system has been cleaned and shaped, pharmacological drugs known as intracanal medicaments are inserted inside the root canal between treatments [4]. The three antibiotic components of a triple antibiotic, an intracanal medicament are minocycline, ciprofloxacin, and metronidazole combined in a 1:1:1 ratio [5]. However, the use of TAP in clinical settings is linked to tooth discoloration because of the minocycline component [6]. The standard TAP can be replaced with a triple antibiotic paste that contains clindamycin, called Modified triple antibiotic paste (MTAP) [7]. TAP powder is transformed into a paste by mixing it with a liquid or gel-like substance, which serves as a carrier. Sterile water, saline, pure glycerine, and chlorhexidine were frequently employed as TAP carriers. Chlorhexidine (CHX) has proven to have antibacterial efficacy against *E. faecalis* at various doses in previous studies [8]. Salt has been utilized as a preservative since ancient times. Himalayan pink salt, extracted from the kherwa district of

Pakistan, is sodium chloride, but it can also include up to 84 other minerals and trace elements [9, 10]. Several other methods for enumerating the number of bacteria in culture have come to light over the years [11]. The plating of serial dilutions and subsequent counting of colony forming units (CFU) method is one of the conventional techniques used for quantitative population evaluation of cultures. Greater intracanal medication efficacy is indicated by fewer live bacteria [12]. Therefore, it is of interest to compare the efficacy of triple antibiotic paste and modified triple antibiotic paste combined with Himalayan pink rock salt and chlorhexidine as intracanal medicaments to eliminate the accumulation of *E. faecalis*.

Materials and Method:

This in-vitro study was performed in the Department of Conservative Dentistry and Endodontics and the Department of Microbiology.

Preparation of tooth samples:

Sixty single-rooted permanent human teeth were extracted for orthodontic reasons with fully developed root apices were collected and stored in saline. Disinfection of tooth samples was achieved by using a 5% sodium hypochlorite solution. The samples were decoronated below the cemento-enamel junction and the apical part was removed to obtain 6mm of the middle third of the root. Internal diameter was enlarged using Gates Glidden drills no. 3. To remove the organic and inorganic debris, tooth specimens were kept in an ultrasonic bath with 17% EDTA and immersed in 5% sodium hypochlorite for 5 minutes, respectively. Traces of chemicals were removed by immersing the specimens in an ultrasonic bath containing distilled water for 5 minutes. The specimens were then sterilized in an autoclave at 121°C for 15 min.

Contamination of the prepared tooth samples:

A few colonies from the culture of *E. faecalis* (ATCC 29212) were inoculated in brain heart infusion (BHI) broth, and incubated at 37 °C for 24 hours. After confirming 0.5 McFarland concentrations, 10 µL inoculum of this culture was introduced into 60 pre-sterilized 150-ml test tubes along with 5 ml of Brain Heart Infusion broth and samples to be infected followed by incubation at 37 °C for 24 hours.

Placement of intracanal medicament:

After 24 hours of incubation, tooth samples were irrigated with 5 mL of sterile saline, divided into 2 groups containing 30 samples each, and assigned to the subgroups (n=15 each) of intracanal medicaments that were placed in the canal.

Group A - Triple antibiotic paste (powder form mixed in 1:1:1)

Group B - Modified triple antibiotic powder (powder form mixed in 1:1:1 ratio)

Subgroup a₁ - Triple antibiotic powder with 2% chlorhexidine

Subgroup a₂ - Triple antibiotic powder and Himalayan pink rock salt with 2% chlorhexidine

Subgroup b₁ - Modified triple antibiotic powder with 2% chlorhexidine

Subgroup b₂ - Modified triple antibiotic powder and Himalayan pink rock salt with 2% chlorhexidine.

Himalayan pink rock salt was combined with the antibiotic powder in subgroup a₂ and subgroup b₂ in a 1:1:1:1 ratio. 1 mg of powder was mixed with 1 ml of 2% chlorhexidine along with methyl cellulose to achieve a paste-like consistency. The intracanal medicaments were placed with lentulospiral (size #2)

after which the apical end of the samples was sealed with 2 coats of transparent nail varnish. Antibacterial evaluation was performed after 24 hours, 3 and 7 days from each group, incubated at 37°C.

Antibacterial evaluation:

The dentinal shavings were collected with GG drill no. 3, transferred to a 150-ml test tube containing 2 ml of BHI broth, and incubated for 24 hours at 37 °C. After incubation, the samples were diluted and coated on a sterile Petri dish consisting of BHI agar. Culture plates were incubated for 24 hours at 37 °C, after which the colonies formed per unit of the culture were manually counted and recorded.

Results:

The data was further statistically analyzed by one-way ANOVA, an independent sample t-test, and a paired sample t-test. Significance was defined by p values less than 0.05. Data analysis was performed using IBM-SPSS version 21.0 (IBM-SPSS Science Inc., Chicago, IL). According to the results obtained, Group B was more effective in eradicating *E. faecalis* than Group A on Day 1 and Day 3, whereas on Day 7, no colonies were found. The mean and standard deviation values for Group A and Group B on Day 1 and Day 3 are presented in Table 1. Among the subgroups, subgroup b₂ showed the best results, followed by subgroups a₂, b₁, and a₁ against *E. faecalis* on day 1 and 3. The mean changes of different subgroups at Day 1 and Day 3 are presented in Table 2. On day 7, all the subgroups showed zero colonies. The bacterial colonies formed in different subgroups at 1 day and 3-day time intervals are given in Figure 1.

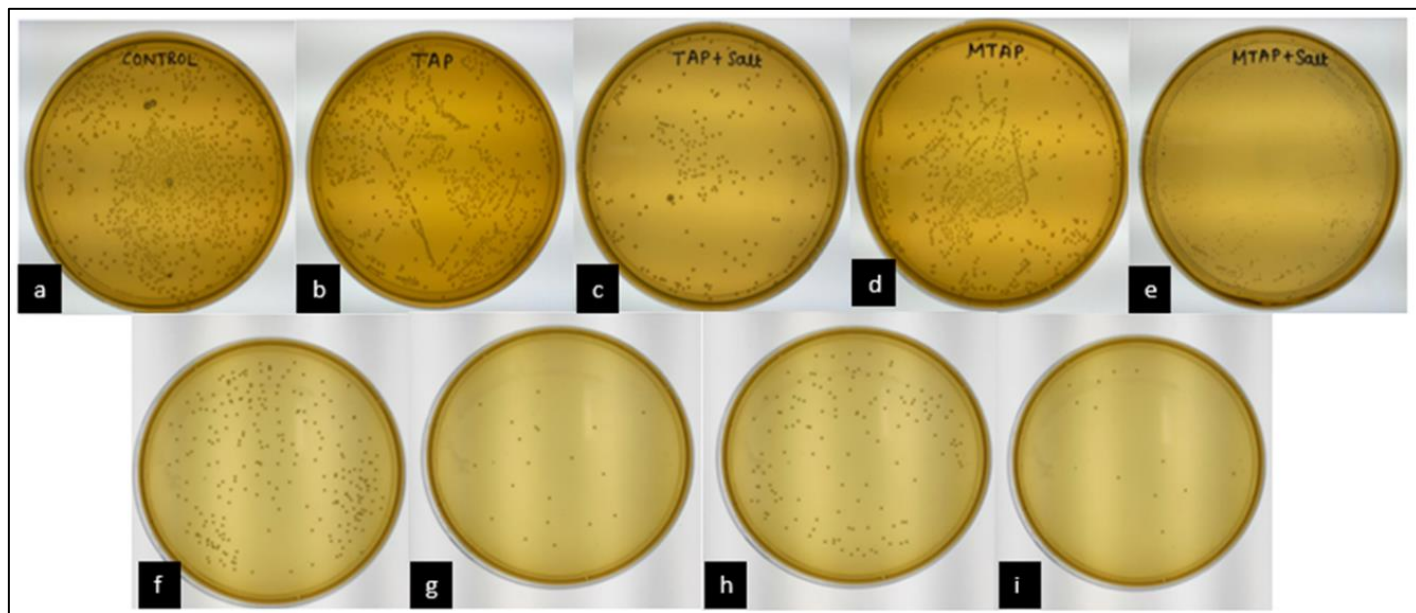


Figure 1: Bacterial colonies on culture plates. (a) Control group after 24 hrs; (b) and (f) Subgroup a₁ after 24 hrs and 3 days; (c) and (g) Subgroup a₂ after 24 hrs and 3 days; (d) and (h) Subgroup b₁ after 24 hrs and 3 days; (e) and (i) Subgroup b₂ after 24 hrs and 3 days.

Table 1: Mean and standard deviation values for Group A and Group B on Day 1 and Day 3

Groups	Mean CFU at Day 1	Std deviation	p	Mean CFU at Day 3	Std deviation	p	Mean CFU at Day 7
Group A	1.72	0.35	0.003	1	0.73	0.024	0
Group B	1.38	0.48		0.6	0.59		0

On intergroup comparison, mean colony forming units for Group A at Day 1 were 1.72, and for Group B, they were 1.38. At Day 3, the mean colony-forming units for Group A were 0.73 and Group B was 0.59. On both days, there was a statistically significant difference between the two groups. An independent sample t test indicates a p value ≤ 0.005 as statistically significant.

Table 2: Mean changes of different subgroups on Day 1 and Day 3

Subgroups pairs	Mean difference in CFU at Day 1	p	Mean difference CFU at Day 3	p	Mean difference in CFU at Day 7
Subgroup a ₁ with a ₂	0.634	<0.0001	1.38	<0.0001	0
Subgroup a ₁ with b ₁	0.192	<0.0001	0.517	<0.0001	0
Subgroup a ₁ with b ₂	1.118	<0.0001	1.651	<0.0001	0
Subgroup b ₁ with a ₂	0.441	<0.0001	0.862	<0.0001	0
Subgroup a ₂ with b ₂	0.484	<0.0001	0.271	<0.0001	0
Subgroup b ₁ with b ₂	0.926	<0.0001	1.134	<0.0001	0

Comparison of mean change in colony forming units between different subgroups at both time intervals (Day 1 and Day 3) is shown. The highest antibacterial effect was seen for subgroup b₂, and the least effect was for subgroup a₁. A pairwise sample t-test indicates a p-value < 0.005 as statistically significant.

Discussion:

The *in vitro* model by Haapasalo & Orstavik is the basis for this study [13]. Due to its failure to capture the largely sclerotic state of apical dentin, this model has obvious shortcomings. The middle section of the canal was chosen to make up for the apical sclerosis [13]. Ciprofloxacin is a fluoroquinolone antibiotic with good tissue penetration and a wide spectrum of activity [14]. The three drugs together in one mixture lower the likelihood of microbial resistance. TAP can induce regeneration, permitting stem cell multiplication in the apical area, leading to the formation of an apical barrier. Metronidazole binds to and destroys DNA in cells. Minocycline inhibits protein synthesis by binding to the 30S ribosome [15]. Clindamycin was added to TAP in place of minocycline to reduce the discoloration caused by binding with collagen [16]. Clindamycin reversibly binds to the 50S ribosomal subunits of bacteria to block the formation of peptide bonds [17]. The results of the present study revealed that the antimicrobial efficacy of MTAP was better than that of TAP. This may be due to the prolonged post-antibiotic effect exerted by clindamycin compared to minocycline, as it binds to the various proteins in the bacteria, causing cell death [18]. Subgroup b₂ revealed the maximum antibacterial efficacy among all. At sub-inhibitory concentrations, clindamycin may boost phagocytosis and decrease the synthesis of toxins. HPRS is composed of 99% sodium chloride. A bacterial cell undergoes a hyperosmotic stress reaction when exposed to a hypertonic salt solution. This leads to a loss of turgor, which results in the loss of intracellular water [19]. Subgroup a₂ was more effective compared to a₁ and b₁, but less effective than b₂. The minimum bactericidal concentration of clindamycin stands at 0.22 µg/ml, whereas for minocycline, it is notably higher at 16 µg/ml [20-21]. Subgroup b₁ was more effective than subgroup a₁. Clindamycin's capacity to reach greater depths within the dentinal tubules, unlike minocycline, contributes to its superior eradication of *E. faecalis* [22]. In our study, subgroup a₁ demonstrated lower effectiveness compared to other treatment groups. Ghabraei *et al.* observed that calcium hydroxide exhibited greater potency compared to TAP in their study. All subgroups exhibited a notable disparity in colony-forming units between day 1 and day 3. By the end of the 7 days, no growth was observed. TAP (Triple Antibiotic Paste) has been reported to exert its antimicrobial

effect over a period ranging from 7 to 21 days [23]. However, more in-vivo trials are needed to fully evaluate these medicaments before their clinical usage.

Conclusion:

Data shows that a modified triple antibiotic mixed with Himalayan pink rock salt can be used as a potential intracanal medicament in combination with 2% Chlorhexidine.

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