Original Article

Comparative evaluation of antimicrobial efficacy of Asphaline Temp, Triple antibiotic Paste and Ultracal XS against Enterococcus faecalis – An *in vitro* study

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ABSTRACT

Aim: This study aimed to evaluate and compare the antimicrobial efficacy of asphaline temp, triple antibiotic paste (TAP), and Ultracal Xs against *Enterococcus faecalis* – An *in vitro* study.

Materials and method: A hundred and twenty freshly extracted, single-rooted human permanent teeth were instrumented and autoclaved. Samples were inoculated with the pure culture of *E. faecalis* and incubated. After incubation, colony-forming units (CFUs) were recorded before medication. Each group was further divided into three subgroups containing ten samples each for days – 1 day, 5 days, and 7 days. Group A – Asphaline Temp; Group B – TAP; Group C – Calcium hydroxide; and Group D – Normal saline (Control). The various time interval bacterial (CFU) within the group were compared by the Analysis of Variance followed by Tukey's *Post hoc* test.

Results: The greater antimicrobial effects were observed in the samples treated with Asphaline temp ($P < 0.005$). No statistical antimicrobial difference was found between Asphaline temp and TAP.

Conclusion: Asphaline temp demonstrated significant antimicrobial effectiveness against *E. faecalis*.

Keywords: Asphaline temp, calcium hydroxide, *Enterococcus faecalis,* intracanal medicament, triple antibiotic paste

INTRODUCTION

One of the most important goals of endodontic treatment is thorough debridement and reduction of microorganisms from the root canal space to create favorable environment for healing and prevent reinfection for long-term success.[1]

Endodontic infections are considered to be polymicrobial in nature with predominant anaerobic microorganisms;[1] among this *Enterococcus faecalis,* a Gram-positive facultative anaerobic cocci which was considered to be the most prevalent microorganism isolated in chronic periodontitis and failed root canal treatment.[2]

Calcium hydroxide ($Ca[OH]_2$) was introduced in 1920 as a pulp-capping agent, it was also widely used because of its various biological properties such as antimicrobial activity, tissue-dissolving ability, inhibition of tooth resorption, and hard-tissue formation;[3] also, it is widely used as an intracanal medicament because of its alkaline pH that destroys the bacterial cell membrane and protein structures.[4]

Triple antibiotic paste (TAP) is a combination of three antibiotics. Hoshino *et al*. recommended metronidazole (500 mg), minocycline (100 mg), and ciprofloxacin (200 mg) at 1:1:1 ratio for 3 mix formulation. The carrier is propylene glycol and

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macrogol ointment at 1:1 ratio.[5] This formulation was later modified by Takushige *et al*. as metronidazole, minocycline, and ciprofloxacin mixed in a ratio of 3:3:1.[6] Metronidazole is a nitro imidazole compound; selectively toxic and effective against anaerobic organisms. The presence of redox protein reduces the nitro groups of this compound and generates free radicals that cause DNA damage and lysis of cell. Minocycline is a bacteriostatic that inhibits protein synthesis by binding to 30S ribosome in the susceptible organisms. Ciprofloxacin is a synthetic fluoroquinolone with rapid bactericidal action. It inhibits the enzyme bacterial DNA gyrase, but this paste has its own drawback.[5]

Asphaline temp is newly introduced in the market and is an iodine-based intracanal medicament with zinc oxide and barium sulfate. However, the literature does not provide adequate data regarding the new material.

Hence, the study aimed to evaluate and compare the antimicrobial efficacy of Asphaline Temp, TAP, and Ultracal Xs against *E. faecalis*.

MATERIALS AND METHOD

Sample preparation

A hundred and twenty single-rooted human permanent teeth which was scheduled for the extraction due to periodontal and orthodontic purpose with patient's verbal consent were collected and stored in 5.2% sodium hypochlorite (NaOCl) solution for 30 minutes to remove the organic residues and left in saline solution (0.9 w/v NaCl) until the procedure began. All the teeth were decoronated 2–3 mm below the cementoenamel junction using the high-speed diamond disk to obtain standardized root canal length of 15 mm. The coronal third was prepared by Gates-Glidden drills (1, 2, 3, 4) (Mani Inc., Japan and 10 K-file [Mani Inc., Japan]) was introduced into the canal until it appeared at the apical foramen. The working length was established by subtracting 0.5 mm from this length. The canal was shaped with Crown-Down technique up to F3 (30/0.09) with using ProTaper gold rotary files (DENTSPLY Sirona, Ballaigues, Switzerland). The canal was irrigated copiously with saline after each instrumentation with side-vented needle and final rinse with 5 ml of 5.25% NaOCl (Prime Dental Product, Mumbai, Maharashtra, India) followed by 1 mL of 17% ethylenediaminetetraacetic acid (Prime Dental Product) to facilitate the removal of smear layer. The roots were coated with nail varnish and apical foramen sealed with resin-modified glass ionomer cement (GIC; GC Fuji, Tokyo, Japan). The roots were sterilized in an autoclave for 15 min at 121°C at 15lb pressure to ensure complete sterilization within the canal. Efficacy of sterilization was tested by sampling the canal with sterile paper points. The paper points were placed into test tube containing 1 ml transport fluid, vortexed for 10s, placed on Agar (HiMedia Laboratories, Mumbai, Maharashtra, India) incubated at 37°C for 48 h, and growth was examined.

Contamination of samples

A suspension of *E. faecalis* (ATCC 29212) was adjusted to 0.5 turbidity on the McFarland scale (1.5×10^8 bacteria/mL). *E. faecalis* suspension of 10 µL was injected into each group. Then, inoculated specimens with *E. faecalis* were placed in tubes filled with brain–heart infusion (BHI) broth, and then, inoculum was added every day and incubated aerobically at 37°C for 21 days. After incubation, the first microbiological sampling was performed by flooding the canal with sterile saline followed by placing a size 30 Hedstrom file into the canal to scrape the dentin during the process. A sterile paper point was placed in the canal for 60 s and transferred into the test tubes containing 1 ml of saline and shaken for 60 s in a vortex mixer. A serial 10-fold dilution was prepared (up to $1:10^5$), and 0.1 ml was transferred and plated on Agar plate (Hi Media laboratories, Mumbai, Maharashtra, India).

The plates were incubated in an aerobic chamber for 24 h at 37°C. Any bacterial growth was detected by performing colony count which was tabulated for all the 120 samples to establish the level of contamination before the application of intracanal medicament.

Antibacterial assessment

The samples were randomly divided into the following groups ($n = 30$): Group 1: Asphaline Temp (Becht Germany), Group 2: TAP, Group 3: Ultracal Xs (Ultradent products, USA), and Group 4: Saline (Control). A sterile cotton pellet was placed in the entire canal orifice and sealed with tin foil. After the loading of the various medicaments, all the groups were subdivided into three subgroups of ten samples and incubated for different experimental time periods of 1, 5, and 7 days. After incubation for 24 h at 37°C, the 40 samples (ten from each group) were uncovered in an aseptic environment. The colony counts were performed for these samples. These were repeated for the Fifth and seventh day.

The percentage reduction in colony count (%RCC) was calculated by using the following formula:

$$\text{Percentage reduction in colony} = \frac{\text{Initial colony} - \text{Final colony}}{\text{Initial colony}} \times 100$$

Statistical analysis

All four groups were compared for colony-forming unit by

the analysis of variance followed by Tukey's *Post hoc* test for intergroup (one to one) comparison.

For all the above tests, *P* value is considered statistically significant when it was <0.05. The software used was Statistical Package for the Social Sciences software version 17 (IBM, Chicago).

RESULTS

Table 1 shows the %RCC for all the groups at various time intervals. For Asphaline Temp, minimum reduction seen was 69.09, 96.08, and 96.82, and maximum of 82.31, 99.00, 99.00 on 1, 5, and 7 days, respectively. For TAP, minimum reduction seen was 62.50, 86.00, and 93.64 and maximum of 77.69, 92.73, 95.83, 99.00, and 99.00 on 1, 5, and 7 days, respectively. Calcium hydroxide minimum reduction seen was 5.00, 33.00, 50.00, and maximum of 33.08, 53.85, and 73.33 on 1, 5, and 7 days, respectively. For normal saline, minimum reduction seen was 0.09, 0.17, and 0.67 and maximum of 8.00, 4.40, and 8.42 on 1, 5, and 7 days, respectively.

The inhibition of growth of *E. faecalis* at the end of 1, 5, and 7 days varied with different intracanal medicament. Asphaline Temp and TAP showed maximum %RCC compared with calcium hydroxide which showed its maximum antibacterial activity at the Seventh day (59.91%). Moreover, Asphaline temp exhibited maximum antibacterial activity at the Fifth day (97.79) which was insignificant with TAP which showed its maximum antibacterial at the Seventh day (94.92). Saline showed least antibacterial activity (3.071).

DISCUSSION

E. faecalis, a Gram-positive, facultative anaerobic microbe, was chosen in this study because it is most commonly found in posttreatment apical periodontitis and seems to be difficult to eradicate from the root canal system because of the several virulence factor of *E. faecalis* which helps them to survive inside the root canal.[7] The study mimicked clinical conditions by using human teeth with the presence of cementum instead of bovine teeth.[8]

Twenty-one days incubation period allowed the suspension of microorganism to completely diffuse throughout the root canal spaces.[9]

Calcium hydroxide is an effective intracanal medicament, as it has high pH (12.5–12.8) and its antimicrobial effectiveness effect is influenced by the dissociation of hydroxyl ions in the aqueous environment. The hydroxyl ions are highly oxidizing radicals that destroys bacteria by damaging the cytoplasmic membrane, protein denaturation, and damaging bacterial DNA.[10]

In the present study, bactericidal effect of $Ca(OH)_2$ was lower (59.81% on 7 days) when compared with other medicaments. This observation was in agreement with recent studies done by Ravi *et al*.,[11] Madhubala *et al*.,[12] and Chittrarasu *et al*.[13] but contradicts with the findings of Lima *et al*.[14] and Manzur *et al*.[15]

The decreased effect of antimicrobial efficacy might be due to the ability of *E. faecalis* to tolerate the exposure to calcium hydroxide because when the bacteria face an

Table 1: Percentage reduction of bacterial count among four groups at different time interval

	Descriptive statistics				
Group	***n***	**Minimum**	**Maximum**	**Mean percentage**	**SD**
Asphaline temp					
Reduction 1 day	10	69.09	82.31	77.8465	4.52878
Reduction 5 days	10	96.08	99.00	97.7949	0.87430
Reduction 7 days	10	96.82	99.00	98.0180	0.64061
TAP					
Reduction 1 day	10	62.50	77.69	70.9896	5.61531
Reduction 5 days	10	86.00	92.73	90.5749	1.94592
Reduction 7 days	10	93.64	95.83	94.9274	0.72489
Calcium hydroxide					
Reduction 1 day	10	5.00	33.08	22.0668	8.72933
Reduction 5 days	10	33.00	53.85	44.3849	7.09931
Reduction 7 days	10	50.00	73.33	59.8104	7.11961
Normal saline					
Reduction 1 day	10	0.09	8.00	2.2572	2.36027
Reduction 5 days	10	0.17	4.40	2.1921	1.49412
Reduction 7 days	10	0.67	8.42	3.0710	2.56264

SD: Standard deviation, TAP: Triple antibiotic paste

adverse or potentially lethal challenge, a stress response is mounted that allows them to endure the threat, survive, and recover. This response has been described for a diverse range of stresses, and in some bacterial species, the adaptive response may contribute to pathogenesis.[16] Furthermore, the endodontic literature shows discouraging information on the antibacterial effectiveness of calcium hydroxide against *E. faecalis* due to the buffering action of dentin.[17]

TAP showed 94.92% RCC on the Seventh day, with statistical insignificant difference with Asphaline temp which showed 97.79% reduction on the Fifth day and higher antimicrobial efficacy as compared to calcium hydroxide. TAP includes metronidazole, ciprofloxacin, and minocycline. Among this metronidazole has a wide bactericidal spectrum against obligate anaerobes, which resides in the deep dentin of infected canal, certain microbes are resistant to it so ciprofloxacin and minocycline were added to obtain higher antimicrobial efficacy.[18]

Triple antibiotic has shown to completely inhibit *E. faecalis* strain on BHI blood agar.[19] Furthermore, the largest zone of inhibition was seen with this group.[20] According to Jong *et al*., even a short-application period of 24–48 Hours can cause tooth discoloration as minocycline binds to calcium ions by chelation to form an insoluble complex.[21]

In the present study, Asphaline temp has shown maximum reduction against *E. faecalis*. The mechanism of action might be due to its iodine molecule by the virtue of its affinity for cell membrane where it exerts its antibacterial effect.[22] Furthermore, it is active against all microorganisms, including Gram-positive, Gram-negative bacteria, spores, mycobacteria, viruses, protozoa, and fungus. Spores, mycobacteria, fungi, viruses, and protozoa.[23]

Asphaline temp can be the better alternative for intracanal medicament as it is readily available in paste and according to manufacturer's instructions, it is not suppose to be used beyond Five days due to discoloration of tooth.

CONCLUSION

Under the limitations of this study, Asphaline temp showed the maximum bacterial reduction followed by TAP, calcium hydroxide showed the least. There was a significant difference when compared with Asphaline temp and calcium hydroxide, but the difference between Asphaline Temp and Triple antibiotic was not that significant.

More studies need to be conducted with the new product such as efficacy on depth penetration.

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Conflicts of interest

There are no conflicts of interest.

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