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In-Vitro Evaluation of Antimicrobial Efficacy of Triple Antibiotic Paste, Metapex and Newly Introduced Iodine Based Asphaline Temp as Intracanal Medicament Against Enterococcus Faecalis

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***Abstract*---Background**: Microorganisms and their products are considered as the etiological agents of pulp necrosis and peri-radicular lesions. They may survive during endodontic procedures due to anatomical structural complexities and limitations of access by instrumentation and irrigants. The present study was conducted to assess antimicrobial efficacy of newly introduced iodine based Asphaline Temp, Triple antibiotic paste and Calcium hydroxide based Metapex against Enterococcus faecalis. **Materials & Methods**: 90 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)

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were recorded before medication. Each group was further divided into 3 subgroups based on time duration of testing for days – 1 day, 5 days, and 7 days, containing 10 samples each. Group I – Asphaline Temp; Group II – Triple antibiotic paste (TAP); Group III – Metapex. The various time interval bacterial CFUs within the group were compared. **Results**: Percentage reduction in bacteria count on 1 day, 5 day and 7 day in Group I (Asphaline Temp) was 77.2, 96.5 and 98.3; in Group II (TAP) was 70.5, 90.4 and 93.2 and in Group III (Metapex) was 25.8, 46.2 and 58.2.

***Keywords*---**asphaline temp, calcium hydroxide, colony-forming units, intra-canal 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 re-infection for long-term success (Sato et al., 1996). The root canal success mainly depends on mechanical preparation, irrigation, microbial control, and complete filling of the root canal system (Alam et al., 2005). Microorganisms, communities of bacteria and their by-products are considered as the etiological agents of pulp necrosis and peri-radicular lesions. They may survive during endodontic procedures due to anatomical structural complexities and limitations of access by instrumentation and irrigants used during chemo-mechanical preparation. To ensure complete elimination of root canal bacteria, effective antimicrobial agents are required for a predetermined time period for predictable outcome (Adl et al., 2012).

Metapex having calcium hydroxide ($Ca[OH]_2$) in its composition has antimicrobial activity, tissue-dissolving ability, inhibit tooth resorption, and hard-tissue formation properties. Triple antibiotic paste (TAP) is a combination of three antibiotics namely- metronidazole, minocycline and ciprofloxacin (Kim et al., 2010). Metronidazole is a nitro imidazole compound; selectively toxic and effective against anaerobic organisms. Ciprofloxacin is a synthetic fluoroquinolone with rapid bactericidal action. It inhibits the enzyme bacterial DNA gyrase. Asphaline temp is newly introduced in the market and is an iodine-based intracanal medicament with zinc oxide and barium sulfate. Iodine acts as an oxidizing agent that reacts with free sulphydryl groups of bacterial enzymes, depicting potent bactericidal action (Schreier et al., 1997). So, the present in-vitro study was conducted to assess antimicrobial efficacy of Asphaline Temp, TAP, and Calcium hydroxide based Metapex against *E. faecalis*.

Materials and Method

The present study invitro study comprised of 90 extracted, single-rooted human permanent teeth which were instrumented and autoclaved. Samples were inoculated with the pure culture of *E. faecalis* (ATCC 29212) and incubated. After incubation, colony-forming units (CFUs) were recorded before medication. Each group was further divided into 3 subgroups containing 10 samples each for days –

1 day, 5 days, and 7 days. Group I – Asphaline Temp; Group II – TAP; Group III – Metapex. The various time interval bacterial (CFU) within the group were compared. Data thus obtained were subjected to statistical analysis using SPSS 19. Level of significance p value < 0.05 was considered significant.

Results

Table 1
Distribution of samples

Groups	Group I	Group II	Group III
Agent	Asphaline Temp	TAP	Metapex
Samples	30	30	30

Table 1 shows specimen distribution in different groups based on different medicaments studied with each group having 30 samples.

Table 2
Percentage reduction of bacterial count

Groups	Reduction 1 day	Reduction 5 day	Reduction 7 day	p value
Group I	77.2	96.5	98.3	0.03
Group II	70.5	90.4	93.2	0.02
Group III	25.8	46.2	58.2	0.01

Table 2, Figure 1, shows that percentage reduction on bacteria count on 1 day, 5 day and 7 day in Group I was 77.2, 96.5 and 98.3, in Group II was 70.5, 90.4 and 93.2 and in Group III was 25.8, 46.2 and 58.2. The difference was significant (p< 0.05).

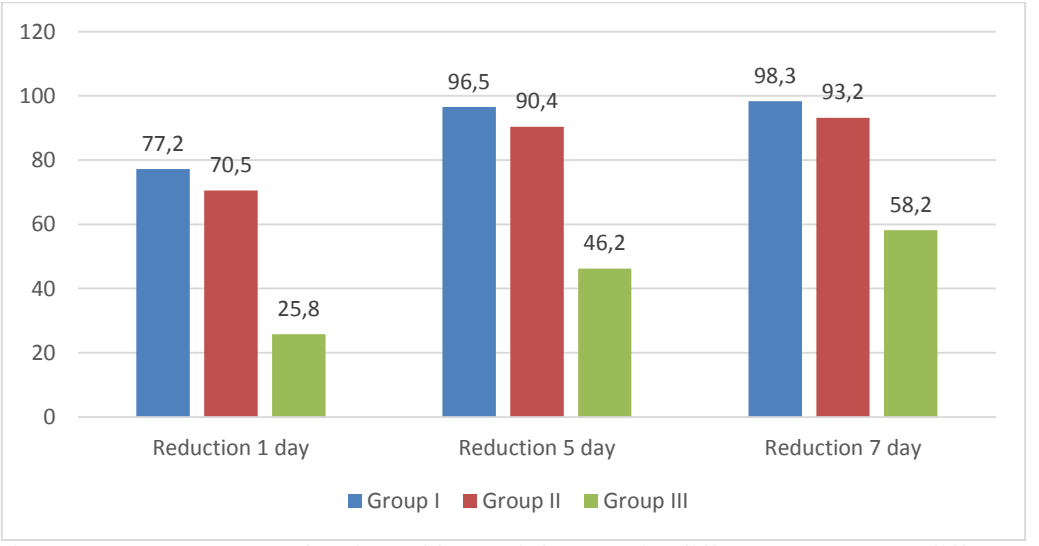

Figure 1. Percentage reduction of bacterial count in different groups at different time periods

Discussion

An ideal endodontic therapy requires complete disinfection of root canal. Many studies have reported that *C. albicans* and *E. faecalis* are able to invade root canal dentinal tubules to variable depth (Cherry et al., 2007). So, to ensure complete elimination of root canal bacteria, an effective antimicrobial agent is required for a predetermined time period in the root canal space (Madhubala et al., 2011). Calcium hydroxide has been widely used as an intracanal medicament in endodontics, and it has been demonstrated that *C. albicans* and *E. faecalis* have reported to be resistant to the antimicrobial effect of calcium hydroxide. Whereas, triple antibiotic paste (TAP) is a mixture of metronidazole, ciprofloxacin, and minocycline (Chittrarasu et al., 2019). TAP possess high anti-microbial efficacy has been effectively used as an intracanal medicament for disinfecting the root canals during regenerative procedures. Therefore, the local application of medicaments within the root canal system may be a more effective mode of delivering anti-microbial drug (Lima et al., 2012). So, the present in-vitro study was conducted to assess antimicrobial efficacy of Asphaline Temp, TAP, and Metapex against E. faecalis.

In present study it was found that Group I having Asphaline Temp showed the maximum bacterial reduction as compared to Group II having TAP and Group III having Metapex. The results were in accordance to the study by Bandekar *et al*[10] who evaluated and compared the antimicrobial efficacy of Asphaline temp, triple antibiotic paste (TAP), and Ultracal Xs against *E. faecalis* and observed greater antimicrobial effects in samples treated with Asphaline Temp ($p<0.005$). However, they found statistically insignificant difference in antimicrobial efficacy between Asphaline Temp and TAP. High antimicrobial efficacy of Asphaline Temp can be attributed to the fact that it predominantly contains Iodine which acts as an oxidizing agent by reacting with free sulphydryl groups of bacterial enzymes, thus having effective bactericidal action (Schreier et al., 1997; Cherry et al., 2007).

Moreover, there was increased percentage reduction in bacteria count at different time intervals in different medicaments studied on 1st day, 5th day and 7th day, which was in Group I 77.2, 96.5 and 98.3 respectively; in Group II was 70.5, 90.4 and 93.2 respectively and in Group III was 25.8, 46.2 and 58.2 respectively. This can be due to the fact that residual microorganism were not able to withstand and recover the antimicrobial stress caused in presence of potent bactericidal agents and could not re-colonize. This was also in accordance to previous study by Shaik et al. (2014), who analyzed the sustained release of intracanal medicaments with or without a carrier at two different time intervals and tested their antimicrobial efficacy in root canal against Candida albicans and Enterococcus faecalis. They concluded that although chitosan was an effective carrier as compared to saline and antimicrobial efficacy increased at different times.

Furthermore, Group II (TAP) showed high percentage reduction on bacteria count of 93.2 on day 7 as compared to Group III that showed 58.2. TAP is predominately composed of metronidazole and minocycline in 1:1 ratio other than ciprofloxacin. Metronidazole is a nitro-imidazole compound that is toxic to anaerobic bacteria cell, thus effective bactericide (Sato et al., 1996; Alam et al., 2005; Adl et al.,

2012; Kim et al., 2010).Whereas, minocycline is a tetracycline derivative having potential antimicrobial property due to its substantivity effect towards microbes (Hoshino et al., 1996). These findings were in accordance to a study by Hoshino et al. (1996), who investigated the antibacterial effect of a mixture of ciprofloxacin, metronidazole, and minocycline with and without the addition of rifampicin on bacteria from infected root canals and concluded that TAP was an effective bactericidal agent when used in combination and alone none of the drugs resulted in complete elimination of bacteria.

Conclusion

The antimicrobial efficacy of different intracanal medicaments can be enhanced by using them in combination as compared to using them alone. Iodine based Asphaline temp showed the maximum bacterial reduction at all time intervals followed by TAP and calcium hydroxide showed the least. So, to avoid certain proven toxic drawbacks of TAP, Asphaline temp provide an effective alternative in eradicating microorganisms of infected root canal for long term success.

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