COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF ODONTOPASTE, CHLORHEXIDINE AND PROPOLIS AS ROOT CANAL MEDICAMENTS AGAINST ENTEROCOCCUS FAECALIS AND CANDIDA ALBICANS

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Abstract

The aim of this study is to compare the antimicrobial efficacy of Odontopaste, Chlorhexidine and Propolis as root canal medicaments against Enterococcus faecalis and Candida albicans.

Under strict aseptic conditions, microbiological sampling is performed using agar diffusion and broth dilution methods for antibacterial sensitivity.

There is a statistically significant difference between three medicaments used against Enterococcus faecalis and Candida albicans.

With-in the limitations of the study, Odontopaste has better antibacterial efficacy against Enterococcus faecalis followed by Chlorhexidine, Propolis shows partial antifungal efficacy against Candida albicans.

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Introduction

Bacteria and their products are considered to be the primary etiological agents of pulpal necrosis and periapical lesions. One of the main goals of root canal treatment is to eliminate bacteria and their by-products before restoration.[1] A favorable outcome of endodontic treatment of teeth with apical periodontitis depends on effective control of root canal infection.[2]

Chemomechanical cleaning and shaping of the root canal can greatly reduce the number of microrganims, but not completely eliminate them.[3]

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Several factors which may contribute to persistent periradicular infection subsequent to root canal treatment include intraradicular infection, extraradicular infection, foreign body reaction and cyst containing cholesterol crystals.[4,5] Studies of dynamics of root canal infections demonstrates that the relative proportions of anaerobic microorganisms and other bacterial cells increase with time, and that the number of facultative anaerobic bacteria 8increases when root canals remain infected for longer periods.[6]

The proportional decrease of the aerobic bacteria and the concomitant increase of strict anaerobic bacteria with time is due to oxygen consumption and a low oxidation-reduction potential. These conditions collaborate to sustain the growth of these bacteria.[7,8]The most resistant strains found frequently in the root canal environment are *Enterococcus faecalis and Candida albicans.*[9] *Enterococcus faecalis* is the most prevalent species isolated from root canals of previously root-filled teeth with chronic apical periodontitis.[10]

Therefore, elimination of this facultative anaerobe cause for an effective antimicrobial treatment protocol to be used to reduce the bacterial insult to minimum allowing host's defencesystem.[11]

Intracanal medicaments are used as an antibacterial agents to eliminate residual bacteria in a root canal after instrumentation and irrigation.[12] They are used to render any remaining canal content inert, to dissolve tissue, act as barrier against leakage or breakdown of the temporary filling, and to control persistent seepage of apical fluids into the root canal system.[12]

This study aimed to evaluate the antimicrobial efficacy of Odontopaste (50 mg or 5% clindamycin hydrochloride, 10 mg or 1% triamcinolone acetonlide and 0.5-1% of calcium hydroxide), Chlorhexidine and Propolis as root canal medicaments used in endodontic therapy against *Enterococcusfaecalis and Candida albicans.*

The antimicrobial activity of the test agents was compared with test agents was compared with the known agents chlorhexidine, with no medicament.

Materials and methods

The materials tested were:

- Odontopaste (Australian Dental Manufacturer OP8G)
- Propolis (O2b Healthy, Newzealand)
- Chlorhexidine (ICPA Health Products, India)- positive control
- No medicament- negative control

The methodology used in this study is a combination of an agar diffusion method and a broth Dilution method.

AGAR DIFFUSION METHOD

The methodology used is adapted from Gomes et al [8]. Standard resistant strains of *Enterococcus faecalis* (ATCC 29212) and *Candida albicans*(ATCC 10239) obtainted for this study. The strains were inoculated and incubated for 37^{o}C for 24 hours in brain heart infusion broth. According to Kriby Bauer's punch well method, the holes were punched in the cultivated agar plates (5mm in depth, 6mm in diameter) and were filled with medicaments.

Each agar plate contained only one medicament. Using sterile tips, 50µl. of antibacterial and antifungal agents were added to the respective wells on the plates.

The lids were closed and the plates incubated at 37^{o}C for 24-48hours. Following the incubation, the diameter of the microbial zones of inhibition around each well was measured and recorded in millimeters.

POUR PLATE METHOD

Fifty single rooted extracted teeth were collected for the present study. Access opening followed by chemomechanical preparation was performed using 18% EDTA (Qualigens Fine Chemicals, Navi Mumbai, India) followed by 5.25% Sodium Hypochlorite (Prime Dental Products, Thane, India).

Each sample was then inoculated with a medicament except for the negative control. The bacterial medium was prepared separately by weighing the ingredients, mixing in the distilled water and autoclaving.

After 24 hours of incubation, dentinal shavings were collected from each sample which were inoculated into the brain heart infusion broth in sterile containers to act as inoculums for testing.

As the sensitivity is tested against five anti-bacterial agents, the medium was prepared in five conical flasks, each contain 100 mg. of cultured medium.

The agar was prepared and sterilized by autoclaving. The culture media were removed from the autoclave, kept on the platforms to allow for cooling to room temperature (around 45^{0} C). The conical flasks were labeled with the respective names of the anti-bacterial and antifungal agents agents.

The sterile glass petri dishes were distributed on level platform. 0.01 ml. of bacterial inoculum was added to all the five conical flasks containing brain heart infusion agar. 100 mg. of each anti-bacterial and antifungal agents agent was added to the respective conical flask.

The medium was mixed thoroughly. 25 ml. of the medium was poured into each petri dish and were allow to solidify at room temperature. The solidified plates were placed in the incubator and incubated at 37^{0}C for 24 – 48 hours. After incubation, these solidified plates were tested for number of colony forming units[9]. (Figure 1-3)

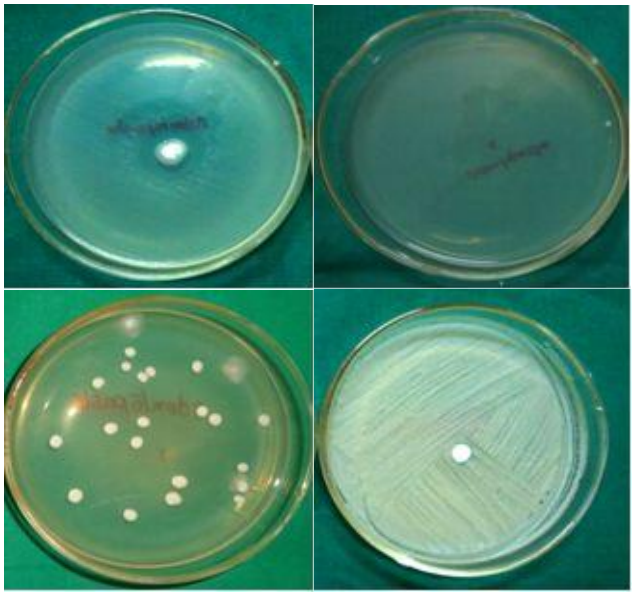


Figure 1. Represents the zone of inhibition and number of colony formings of Odontopaste against *Enterococcus faecalis*and *Candida albicans.*

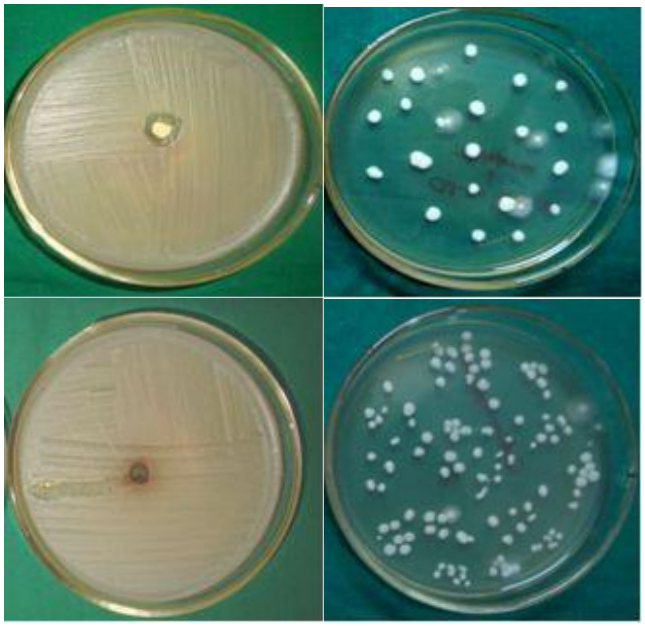


Figure 2. Represents the zone of inhibition and number of colony formings of Chlorhexidine against *Enterococcus faecalis*and *Candida albicans.*

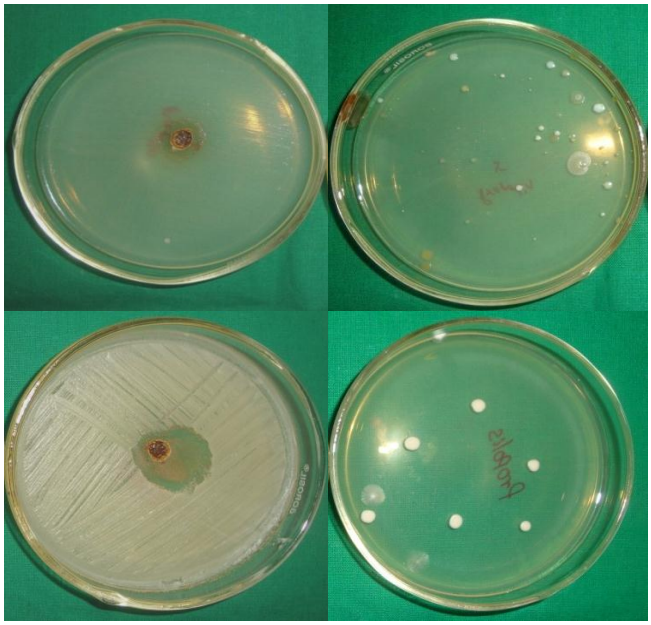


Figure 3. Represents the zone of inhibition and number of colony formings of Propolis against *Enterococcus faecalis* and *Candida albicans.*

Results

These findings were statistically analysed with KruskalWalley test and Man Whitney- U test. It was found that Odontopaste exhibited better antibacterial efficacy than Chlorhexidine and Propolis demonstrates only partial antifungal efficacy.

The microbial zone of inhibition (mm) and number of colony forming units (CFU ml^{-1}) were significantly lower for Odontopaste than other groups. The mean and standard deviation values for the zone of inhibition is shown in Table 1-15 and the number of colony forming units in Table-2.

AGAR DIFFUSION METHOD

Mean values of microbial, zone of inhibition (mm) produced by different intracanal medicaments against *Enterococcus faecalis* and *Candida albicans* were shown in Table1 and 2. All the medicaments produced inhibitory zones ranging from26±8mm against these microorganisms after 24 hours of incubation. However, Odontopaste alone demonstrated the strongest antibacterial action, showing the largest inhibitory growth zones. This was followed by Chlorhexidine which had a smaller inhibitory zones against *Enterococcus faecalis*, where as Propolis demonstrated smaller inhibitory zones against *Candida albicans.*

Table 1 and 2. Means of microbial inhibitory zones (in mm) – Mean and Standard deviation. Diffused method –comparison of products.

Product	24 hurs			48 hours		
	Means	Std.Dev.	Median	Means	Std.Dev.	Median
Odontopaste	23.10	1.79	23.50	20.40	1.51	20.50
Propolis	11.90	1.60	12.00	9.10	0.74	9.00
Chlorhexidine	4.80	1.03	4.50	1.60	0.52	2.00
Negative control	14.50	1.43	14.50	14.00	1.15	14.00

Table 1. Summary statistics**-** anti bacteria effect.

Products	Means	Std.Dev.	Median	Sum of ranks	H-value	P-value
Odontopaste	23.10	1.79	23.50	555.00	55.7374	0.0000
Propolis	11.90	1.60	12.00	365.50		
Chlorhexidine	4.80	1.03	4.50	198.00		
Negative control	14.50	1.43	14.50	444.50		

Table 2. Comparison of four products with respect to anti bacteria effect at 24 hours (**Diffused method**) by Kruskal Wallis ANOVA test by ranks. (*p<0.05)

Products	Means	Std.Dev.	Median	Sum of ranks	U-value	Z-value	P-value
Odontopaste	23.10	1.79	23.50	155.00	0.00	-3.8055	0.0001
Propolis	11.90	1.60	12.00	55.00			
Odontopaste	23.10	1.79	23.50	155.00	0.00	-3.8245	0.0001
Chlorhexidine	4.80	1.03	4.50	55.00			
Odontopaste	23.10	1.79	23.50	155.00	0.00	-3.8011	0.0001
Negative control	14.50	1.43	14.50	55.00			
Propolis	11.90	1.60	12.00	155.00	0.00	-3.8304	0.0001
Chlorhexidine	4.80	1.03	4.50	55.00			
Propolis	11.90	1.60	12.00	65.50	10.50	-3.0378	0.0024
Negative control	14.50	1.43	14.50	144.50			
Chlorhexidine	4.80	1.03	4.50	55.00	0.00	-3.8260	0.0001
Negative control	14.50	1.43	14.50	155.00			

Table 3. Pair wise comparison of four products with respect to respect to anti bacteria effect at 24 hours (**Diffused method**) by Mann-Whitney U test.

Products	Means	Std.Dev.	Median	Sum of ranks	H-value	P-value
Odontopaste	20.40	1.51	20.50	555.00	57.8075	0.0000*
Propolis	9.10	0.74	9.00	355.00		
Chlorhexidine	1.60	0.52	2.00	155.00		
Negative control	14.00	1.15	14.00	455.00		

Table 4. Comparison of four products with respect to anti bacteria effect at 48 hours (**Diffused method**) by Kruskal Wallis ANOVA test by ranks. (*p<0.05)

Products	Means	Std.Dev.	Median	Sum of ranks	U-value	Z-value	P-value
Odontopaste	20.40	1.51	20.50	155.00	0.00	-3.8245	0.0001
Propolis	9.10	0.74	9.00	55.00			
Odontopaste	20.40	1.51	20.50	155.00	0.00	-3.8543	0.0001
Chlorhexidine	1.60	0.52	2.00	55.00			
Odontopaste	20.40	1.51	20.50	155.00	0.00	-3.8055	0.0001
Negative control	14.00	1.15	14.00	55.00			
Propolis	9.10	0.74	9.00	155.00	0.00	-3.8832	0.0001
Chlorhexidine	1.60	0.52	2.00	55.00			
Propolis	9.10	0.74	9.00	55.00	0.00	-3.8333	0.0001
Negative control	14.00	1.15	14.00	155.00			
Chlorhexidine	1.60	0.52	2.00	55.00	0.00	-3.8633	0.0001
Negative control	14.00	1.15	14.00	155.00			

Table 5. Pair wise comparison of four products with respect to respect to anti bacteria effect at 48 hours (**Diffused method**) by Mann-Whitney U test.

Product	24 hurs			48 hours		
	Means	Std.Dev.	Median	Means	Std.Dev.	Median
Odontopaste	0.00	0.00	0.00	0.00	0.00	0.00
Propolis	16.60	1.78	17.00	15.70	0.82	15.50
Chlorhexidine	1.00	0.67	1.00	0.60	0.52	1.00
Negative control	10.60	1.07	11.00	14.90	0.88	15.00

Table 6. Summary statistics anti fungal effect.

Products	Means	Std.Dev.	Median	Sum of ranks	H-value	P-value
Odontopaste	0.00	0.00	0.00	115.00	56.7545	0.0000
Propolis	16.60	1.78	17.00	555.00		
Chlorhexidine	1.00	0.67	1.00	235.00		
Negative control	10.60	1.07	11.00	453.00		

Table 7. Comparison of four products with respect to anti fungal effect at 24 hours (**Diffused method**) by Kruskal Wallis ANOVA test by ranks. (*p<0.05)

Products	Means	Std.Dev.	Median	Sum of ranks	U-value	Z-value	P-value
Odontopaste	0.00	0.00	0.00	55.00	0.00	-4.0489	0.0001
Propolis	16.60	1.78	17.00	155.00			
Odontopaste	0.00	0.00	0.00	65.00	10.00	-3.4733	0.0005
Chlorhexidine	1.00	0.67	1.00	145.00			
Odontopaste	0.00	0.00	0.00	55.00	0.00	-4.0612	0.0000
Negative control	10.60	1.07	11.00	155.00			
Propolis	16.60	1.78	17.00	155.00	0.00	-3.8423	0.0001
Chlorhexidine	1.00	0.67	1.00	55.00			
Chlorhexidine	1.00	0.67	1.00	55.00	0.00	-3.8528	0.0001
Negative control	10.60	1.07	11.00	155.00			

Table 8. Pair wise comparison of four products with respect to respect to anti fungal effect at 24 hours (**Diffused method**) by Mann-Whitney U test.

Products	Means	Std.Dev.	Median	Sum of ranks	H-value	P-value
Odontopaste	0.00	0.00	0.00	125.00	55.0941	0.0000*
Propolis	15.70	0.82	15.50	528.00		
Chlorhexidine	0.60	0.52	1.00	215.00		
Negative control	14.90	0.88	15.00	482.00		

Table 9. Comparison of four products with respect to anti fungal effect at 48 hours (**Diffused method**) by Kruskal Wallis ANOVA test by ranks. (*p<0.05)

Products	Means	Std.Dev.	Median	Sum of ranks	U-value	Z-value	P-value
Odontopaste	0.00	0.00	0.00	55.00	0.00	-4.0825	0.0000
Propolis	15.70	0.82	15.50	155.00			
Odontopaste	0.00	0.00	0.00	75.00	20.00	-2.8536	0.0043
Chlorhexidine	0.60	0.52	1.00	135.00			
Odontopaste	0.00	0.00	0.00	55.00	0.00	-4.0700	0.0000
Negative control	14.90	0.88	15.00	155.00			
Propolis	15.70	0.82	15.50	155.00	0.00	-3.8832	0.0001
Chlorhexidine	0.60	0.52	1.00	55.00			
Propolis	15.70	0.82	15.50	128.00	27.00	-1.8304	0.0672
Negative control	14.90	0.88	15.00	82.00			
Chlorhexidine	1.00	0.67	1.00	55.00	0.00	-3.8725	0.0001
Negative control	10.60	1.07	11.00	155.00			

Table 10. Pair wise comparison of four products with respect to respect to anti fungal effect at 48 hours (**Diffused method**) by Mann-Whitney U test.

	Anti bacterial effect			Anti fungal effect		
Product	Means	Std.Dev.	Median	Means	Std.Dev.	Median
Odontopaste	25700	3401	25500	11000	2357	10500
Propolis	106000	10750	110000	563000	54985	545000
Chlorhexidine	223000	17670	225000	398000	25298	380000
Negative control	878000	18738	875000	783000	24060	775000

Table 11. Summary statistics.

Products	Means	Std.Dev.	Median	Sum of ranks	H-value	P-value
Odontopaste	25700	3401	25500	55.00	57.3700	0.0000*
Propolis	106000	10750	110000	155.00		
Chlorhexidine	223000	17670	225000	354.50		
Negative control	878000	18738	875000	555.00		

Table 12. Comparison of four products with respect to anti bacteria effect (Enterococcus Faecalis in Pour plate method) by Kruskal Wallis ANOVA test by ranks. (*p<0.05)

Products	Means	Std.Dev.	Median	Sum of ranks	U-value	Z-value	P-value
Odontopaste	25700	3401	25500	55.00	0.00	-3.8055	0.0001
Propolis	106000	10750	110000	155.00			
Odontopaste	25700	3401	25500	55.00	0.00	-3.7925	0.0001
Chlorhexidine	223000	17670	225000	155.00			
Odontopaste	25700	3401	25500	55.00	0.00	-3.7954	0.0001
Negative control	878000	18738	875000	155.00			
Propolis	106000	10750	110000	55.00	0.00	-3.8040	0.0001
Chlorhexidine	223000	17670	225000	155.00			
Propolis	106000	10750	110000	55.00	0.00	-3.8069	0.0001
Negative control	878000	18738	875000	155.00			
Chlorhexidine	223000	17670	225000	55.00	0.00	-3.7939	0.0001
Negative control	878000	18738	875000	155.00			

Table 13. Pair wise comparison of four products with respect to respect to anti bacteria effect (Enterococcus Faecalis in Pour plate method) by Mann-Whitney U test.

Products	Means	Std.Dev.	Median	Sum of ranks	H-value	P-value
Odontopaste	11000	2357	10500	55.00	57.4866	0.0000*
Propolis	563000	54985	545000	454.50		
Chlorhexidine	398000	25298	380000	255.00		
Negative control	783000	24060	775000	555.00		

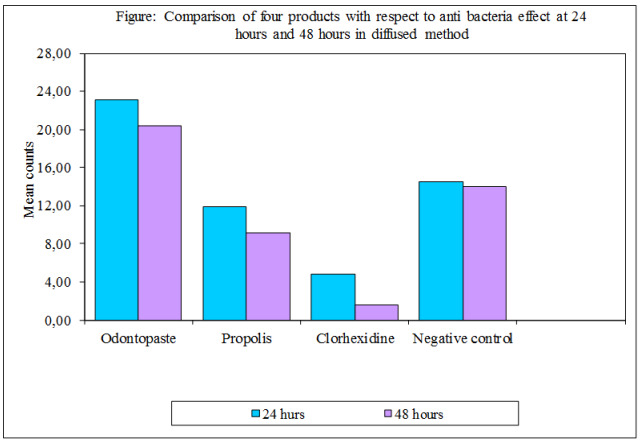
Table 14. Comparison of four products with respect to anti fungal effect (Enterococcus Faecalis in Pour plate method) by Kruskal Wallis ANOVA test by ranks. (*p<0.05)

Products	Means	Std.Dev.	Median	Sum of ranks	U-value	Z-value	P-value
Odontopaste	11000	2357	10500	55.00	0.00	-3.7954	0.0001
Propolis	563000	54985	545000	155.00			
Odontopaste	11000	2357	10500	55.00	0.00	-3.8423	0.0001
Chlorhexidine	398000	25298	380000	155.00			
Odontopaste	11000	2357	10500	55.00	0.00	-3.7954	0.0001
Negative control	783000	24060	775000	155.00			
Propolis	563000	54985	545000	155.00	0.00	-3.8408	0.0001
Chlorhexidine	398000	25298	380000	55.00			
Propolis	563000	54985	545000	55.00	0.00	-3.7939	0.0001
Negative control	783000	24060	775000	155.00			
Chlorhexidine	398000	25298	380000	55.00	0.00	-3.8408	0.0001
Negative control	783000	24060	775000	155.00			

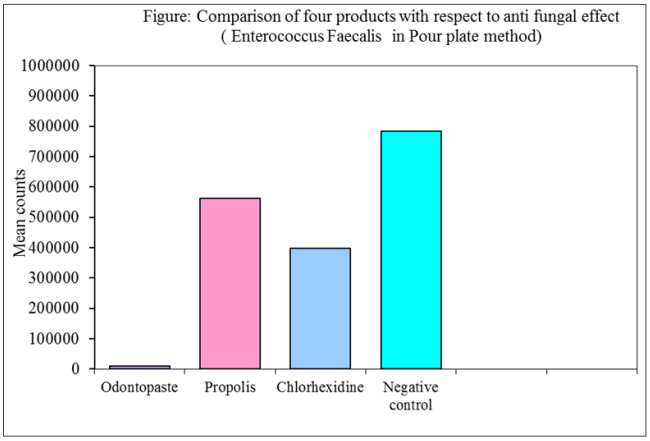
Table 15. Pair wise comparison of four products with respect to respect to anti fungal effect (Enterococcus Faecalis in Pour plate method) by Mann-Whitney U test.

POUR PLATE METHOD

Mean values of microbial Colony forming units (CFU ml^{-1}) produced by different intracanal medicaments against *Enterococcus faecalis and Candida albicans* were shown in Table 5. All these medicaments produced colony forming units ranging from $2.57x10^4$**±**1.65 $x10^5$ against *Enterococcus faecalis and for Candida allbicans* it is rainging from $1.10x10^5$**±**3.98 $x10^5$ after 24 hours of incubation.However, Odontopaste demonstrated the strongest antibacterial action, showing the less number of colonies in the petri dishes followed by Chlorhexidine, Propolis. Propolis alone demonstrated better antifugal action towards other medicaments.



Diffused method –comparison of four products.



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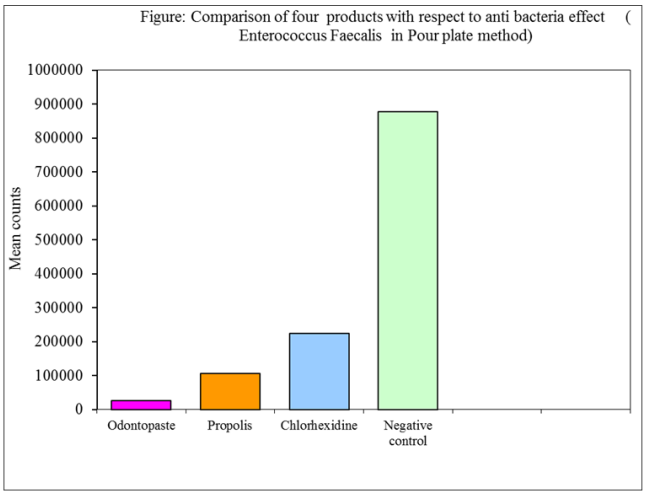
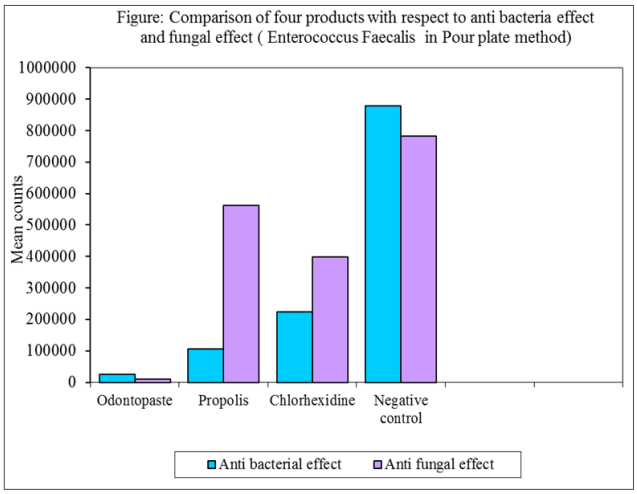


Diffused method –comparison of four products.



Diffused method –comparison of four products.

Discussion

Enterococcus faecalis has demonstrated the capacity to survive in any harsh, stressful environment, including intracellular survival in macrophages. *Enterococcus faecalis* is capable of entering and recovering from the viable but not cultureable (VBNC). VBNC displays cell wall alterations that might provide protection under unfavourable environmental condition.[13]

It has been hypothesized that in periapical infections involving *Enterococcus faecalis,* tissue damage is caused predominantly by host response to the bacteria rather than direct damage from the bacterial products.[14] Love postulated that a virulence factor of *Enterococcus faecalis* in failed endodontically treated teeth may be related to the ability of *Enterococcus faecalis* cells to maintain the capability to invade dentinal tubules and adhere to the collagen in presence of human serum.[15]

Enterococcus faecalis can coaggregates with other species and helps in formation of biofilms. Virulence factors with the potential to promote adaptation and survival in different environments include Enterococcus surface protein (Esp), Collagen binding protein (Ace), proteases and toxins (cytolysin). *Enterococcus faecalis* has potential clinical significance as genes relating to antibiotic resistance, as well as virulence traits, can be found on plasmids that respond to pheromones. Pheromones

from *Enterococcus faecalis* were chemotactic for human neutrofils.[16]

Candida albicans is a dimorphic pathogenic fungus frequently encountered as a commensal of the human digestive system and vaginal tract. *Candida albicans* infections have increased dramatically during the last two decades due to several factors includes immunosuppressive treatments, long-term catheterization, broad-spectrum antibiotic use, and longer survival of immunologically compromised individuals. The infections pro- duced range from the superficial to the systemic. Another interesting feature of C. albicans is its ability to grow in at least two different morphological forms; either as a mycelium or as a yeast cell. Such a transition is induced in response to several environmental conditions, such as the pH or temperature, or different cornpounds, such as N-acetylglucosamine, proline or serum. *Candida albicans* diploidy was deduced from the determination of DNA content2n3 and the kinetics of reassociation of denatured total DNA. In addition, it was found that many clinical isolates of C. albicans displayed a strongly biased spectrum of auxotrophic mutants following ultraviolet (UV) light irradiation,"s33n7, a fact that was interpreted as the consequence of the natural heterozygosity of many *Candida albicans* strains for some loci and the induction of mitotic crossing-over by UV irradia- tion. This hypothesis was confirmed later from the analysis of either sectored colonies, obtained after UV treatment of putative natural heterozygotes, or revertants of mutants isolated by chemical mutagenesis.[17,18]

Odontopaste (Australian Dental Manufacturer) is an intracanal medicament in combination of 50 mg or 5% clindamycin hydrochloride, 10 mg or 1% triamcinolone acetonlide and 0.5-1% of calcium hydroxide. Clindamycin hydrochloride inhibits peptide bond formation in the bacterial DNA and leads to cell death. *Enterococcus faecalis* is intrinsically resistant to clindamycin hydrochloride with a reported minimum inhibitory concentration of 4-16 micrograms.[19] However, the concentration of clinamycin hydrochloride in odontopaste is, approximately 50,000 micrograms per ml, making it effective against *Enterococcus faecalis*. The advantage of the local application of an antibiotic is that it allows for the use of very large doses, hence overcoming resistance, without risk of toxicity to the subject, as the overall dose is small. [20]

In addition, there is the added advantage of the interaction of clindamycin hydrochloride with zinc oxide, since the antibiotic is released slower, hence maintaining a larger concentration within the root canal. Triamcinolone acetonlide is a corticosteroid and reduces the inflammation by inhibiting the activity of mastcells, macrophages and mediators for allergic reactions. Calcium hydroxide's antimicrobial activity is due to release of hydroxyl ions. These are highly oxidant free radicals that show extreme reactions with several biomolecules. This reaction is high and indiscriminate. It may directly affect bacterial cells in three ways; damaging the the bacterial cytoplasmic membrane, protein denaturation and damage to the DNA.[21,22]

However, Australian Dental Manufacturer does not claim any anti-bacterial effect of the calcium hydroxide added and that the function of the calcium hydroxide is to improve the consistency of the paste[23].

Chlorhexidine (CHX) is a broad-spectrum antimicrobial agent that has been reported to be an effective medicament in endodontic therapy. It acts by adsorbing onto the cell wall of the microorganism and causing leakage of intracellular components. At low concentrations of chlorhexidine small molecular weight substances will leach out, resulting in a bacteriostatic effect.[24] The bacteriostatic effect of chlorhexidine is considered to be more important, as the

bounded chlorhexidine molecule is slowly released for upto 24 hours as the concentration decreases. At higher concentrations, chlorhexidine is bactericidal because of precipitation or coagulation of cytoplasm, probably caused by protein cross-linking. CHX may alsoimpart substantive antibacterial activity to root dentin after prolonged exposure(i.e atleast 1 week) [25].

Propolisis a natural premier preventive flavonoid-rich resinous productof honeybees. Its known for its biological properties, including antibacterial, antifungal, antiviral and healing properties.[26]

Krell in 1996 introduced Propolis into dentistry. This product contains vegetable balsams 50%, waxes 30%, essential oils 10%, pollen 5%. The anti-bacterial effect of Propolis is due to the presence of caffeic acid. The release of caffeic acid phenythyl esterase enzyme produces a scavenging effect against bacteria[27.]

The majority of the research in calcium hydroxide antimicrobial activity uses the agar diffusion method, which indicates the medicament's potential to eliminate microorganisms. Clinically the effectiveness of the material is generally reduced by the buffering effect of dentin, the amount of medication placed is usually smaller than that used in in vitro studies, the polymicrobial nature of endodontic infections is difficult to reproduce in vitro, and the presence of biofilms which might require more time and volume of medicaments to exert the same antimicrobial activity. In contrast, the pour plate method is effective when one microorganism is tested to several agents.[28]

Despite the antimicrobial properties of the chemomechanical preparation and the intracanal dressings, the elimination of the microrganisms may not be uniform due to the varying vulnerabilities for the involved species.[29]

Furthermore, the anatomical complexities of many root canals and consequent limitations of access by instruments, irrigants, and intracanal medications are well recognized factors.[30]

Bystrom and Sundquist (1981) verified that bacteria that survived instrumentation. The irrigation quickly proliferated and recolonized root canals that remained empty between treatment sessions.[31]

Siqueira and Uzeda (1996) found that dentin has buffering potential, as in the proton donor that occurs in hydroxyl apatite hydrated layer can reduce the pH effect inside the dentinal tubules. This mechanism is associated with the resistance of *Enterococcus faecalis* to calcium hydroxide.[32]

Gomes and Souza (2003) concluded that 2% Chlorhexidine gel alone is effective against *Enterococcus faecalis*.[33]

Athanassiadis B and Walsh (2008) stated without testing, that *Enterococcus faecalis* acquired high level resistance to clindamycin hydrochloride found in Odontopaste. They consider the paste as a whole; as the large local concentration of clindamycin hydrochloride is in excess of the minimal inhibitory concentration of *Enterococcus faecalis;* they did not consider the possible synergistic effects of Odontopaste's other ingredients additionally they did not consider the physical properties of the paste including the high pH. Their comments were based purely on the pharmacopeia of clindamycin hydrochloride, with no consideration for the uniqueness of its dose of application [34].

Lama Awawdeh and Mohammad Hammad (2009) verified that the apparent use of the natural bee product Propolis is more effective than calcium hydroxide ex vivo in eliminating *Enterococcus faecalis*-within one day[16].

Kandaswamy (2010) verified that 2% Chlorhexidine gel demonstrated better antimicrobial efficacy than propolis, morindacitrifolia and calcium hydroxide[35].

The present study found that the antibacterial activity of Odontopaste was more effective than Chlorhexidine followed by Propolisagainst *Entercoccusfaecalis*.

Propolis was found to be partially effective against *Candida albicans*than other medicaments.

Conclusions

With-in the limitations of present in vitro study, Odontopaste is the most effective antibacterial agent of the medicaments tested against *Enterococcus faecalis*. Propolis is partially effective against *Candida albicans*. Further studies need to be done regarding on the physical properties and pH levels of the medicaments.

Declaration of Interest

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