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Research Article

COMPARISON OF ANTIBACTERIAL EFFICACY OF OCTENIDINE DIHYDROCHLORIDE AND CALCIUM HYDROXIDE, TRIPLE ANTIBIOTIC PASTE, TURMERIC AND CALCIUM HYDROXIDE AS ANINTRACANAL MEDICAMENTSAGAINST ENTEROCOCCUS FAECALIS: AN IN-VITRO STUDY

Manisha Choudary T., Bharat Deosarkar and Priti A Thakare*

Department of Conservative Dentistry & Endodontics, Saraswati Dhanwantari Dental College &Hospital, Parbhani, Maharashtra, India

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ABSTRACT

Aim: The aim of this in vitro study is to compare the antibacterial efficacy of Octenidine Dihydrochloride and Calcium hydroxide, Triple Antibiotic Paste, Turmeric and Calcium hydroxide as anintracanal medicament against Enterococcus faecalis.

Material & Method: The samples were divided into 3 groups. Group A: Octenidinedihydrochloride+ Calcium hydroxide, Group B: Triple Antibiotic Paste, Group C : Turmeric +Calcium hydroxide. Enterococcus faecalis are grown in brain heart infusion broth (BHI). Three wells 8mm diameter were punched in each media plate and filled with respective groups of medicaments. After incubation period of 24 hr, at 37º C, inhibition zones are measured with vernier caliper.

Results: The Triple antibiotic paste showed highest zone of inhibition than other groups.

Conclusion: Under the limitation of this study, all the materials showed antibacterial activity against Enterococcus faecalis but Group B(Triple Antibiotic Paste) showed best efficacy against E. faecalis.

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INTRODUCTION

One of the goals of endodontic treatment is to reduce or eliminate the bacteria and their by-products from the root canal system. Proper cleaning, shaping, and irrigation have been shown to significantly reduce and sometimes eliminate bacteria from canals. To disinfect the root canal system, the use of intracanal medicaments has been advocated.[1]

Enterococcus faecalis is an anaerobic Gram-positive bacteria. It is responsible for 80-90% of Enterococcal infections and the only Enterococcus species isolated from failed obturated root canals. E. faecalis plays an important role in persistent failure of endodontic treatments and it's prevalence in such infection ranges from 24 to 77%. The virulence of E. faecalis may be due to its resistance to conventional intracanal medicaments and its survival in the root canal as a single organism without the support of other bacteria.[2]

Calcium hydroxide is one of the most commonly used intracanal medicament and it eliminates most microorganisms due to it's high p^H(12.5). It is bactericidal and neutralizes the remaining tissue debries in root canal system.[3]It possesses many of the ideal properties of root canal dressing, acting as a physical barrier, preventing root canal reinfection and interrupting the nutrient supply to the remaining bacteria. Other alternative intracanal medicaments used are chlorhexidine, propolis, aloe vera and mushroom.[4]

Herbal products have been used since ancient times in folk medicine, involving both eastern and western medicinal traditions. Many plants with biological and anti-microbiological properties have been studied since there has been a relevant increase in the incidence of antibiotic overuse and misuse.[5]

In the search of a novel antimicrobial compound, traditional plants have proved to be a better source. Highly antimicrobial, anti-inflammatory, antioxidant and biocompatible properties make their use in dentistry more extensive. Judicious use of these herbal formulations has been found to mitigate the E. faecalis count, which could in turn reduce its post-treatment

Corresponding author:* **Priti A Thakare
Department of Conservative Dentistry & Endodontics, SaraswatiDhanwantari Dental College &Hospital, Parbhani, Maharashtra, India

infections. Hence, it can be considered as one of the possible alternatives or a replacement for the synthetic chemical formulations.[6]

Amixture of metronidazole, ciprofloxacin and minocycline known as the Triple Antibiotic Paste (TAP), has been used as an intracanal medicament. Metronidazole is a broad spectrum bactericidal antibiotic. In vitroexperiments have shown that in the infected root canals, 10 µg/ml metronidazole can eliminate more than 99% of bacteria. On the other hand, increasing the concentration of metronidazole could not kill all the bacteria. Therefore, to sterilize the infected root canal, we need other antibiotics such as ciprofloxacin and minocycline. Some researchers have found that the Triple Antibiotic Paste can sterilize root dentin. Also, Adl *et al* showed that TAP can effectively eliminate E. faecalis.[7]

Octenidine hydrochloride (OCT) Octenidine (Schulke and Mayr GmbH, Norderstedt, Germany), is a bispyridinederivative, that is, N, N‑[1,10‑decanediyldi‑(4H)‑pyridinyl‑4pylidene] bis (1‑ octanamine) dihydrochloride. A mouthrinse containing 0.1% OCT may be capable of exerting beneficial clinical effects upon plaque accumulation and gingivitis. To inhibit dental plaque and caries both in rats and human, Octenidine isused in the form of mouthrinse. As a means for prolonged bacterial anti- adhesive activity, it has been revealed that OCT appears to be more effective than chlorhexidine.[8]Hence, the aim of the present study was to compare antibacterial activity of Octenidine Dihydrochloride and Calcium Hydroxide, Triple Antibiotic Paste, Turmeric and Calcium Hydroxide against Enterococcus faecalis.

MATERILS AND METHOD

- OctenidineDihydrochloride
- Triple Antibiotic Paste (Ciprofloxacin, Metronidazole, Minocycline)
- Turmeric
- Normal Saline
- Brain Heart Infusion Broth
- Enterococcus faecalis (ATCC 29212) culture
- Blood Agar

Preparation of Octenidine Dihydrochloridesolution*:*
Octenidinedihydrochloride powder (0.2gm) is mixed with 10ml of distilled water and then add 1gm Calcium Hydroxide to produce a paste.

Preparation of Triple Antibiotic Paste (TAP)*:* Triple Antibiotic Paste (0.5g of ciprofloxacin + 0.5g of minocycline + 0.5g of metronidazole) was mixed with 0.5ml of normal saline by 1:1:1 proportion to produce paste.

Preparation of Aqueous Solution of Turmeric*:* Curcuma longa commonly known as turmeric, is an herbaceous perennial plant.

The rhizomes were washed with distilled water and then cut into irregular large pieces. They were then dried in an oven by tray drying process at a temperature of 45±5° C for a period of about 10 days till they were completely moisture-free. These irregular large size pieces were ground to form a coarse powder.

Maceration process of extraction was then performed to obtain course powder of rhizome. 25 gms of coarse powder of the Curcuma longa rhizome and 25 gms of calcium hydroxide powder was placed in a large glass chamber.

100 ml of sterile distilled water was added to a glass chamber prepare the paste. The glass chamber was closed with a glass lid to prevent evaporation of the menstruum and this system was allowed to stand for 7 days with occasional stirring. The liquid obtained was stored in a refrigerator at 4°C in a beaker.

Test For Antibacterial Assay*:* A loopful of bacterial colonies of Enterococcus faecalis was mixed with 1ml dialyzed brain heart infusion broth (equivalent to 0.5 mcfarland unit) and was poured and spread with sterile glass bead on prepared agar plates (Figure 1).

They are divided as Group A, B, C

Group A – OctenidineDihydrochloride + Calcium Hydroxide
Group B – Triple Antibiotic Paste
Group C – Turmeric + Calcium Hydroxide

Agar Diffusion Method

To check antibacterial efficacy of Octenidinedihydrochloride, Triple antibiotic paste and Turmeric agar diffusion method was performed. Three round well 4 mm deep and 8mm diameter were made in each plate using sterile cork borer (Figure 2). Octenidinedihydrochloridepaste, Triple antibiotic paste and Turmeric 100microlitreeach was added to respective wells and plates were incubated for 24 hr at 37º C in an incubator.

After incubation period, plates were removed and zone of inhibition (Figure 3) will be measured with vernier caliper.

Fig 1 E. faecalis cultured on blood agar

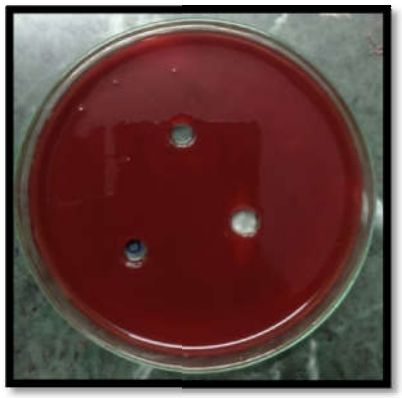


Fig 2 Three round well of group A, B ,C

Fig 3 Zone of inhibition of Group A, B ,C

RESULT

Results were tabulated and statistically analyzed using one way analysis of variance (ANOVA)followed by Tukeys multiple posthoc test.

Table 1 Mean Zone of Inhibition (in mm) of different experimental groups.

Groups	Zone of inhibition (in mm)
Group A (Octenidinedihydrochloride + Calcium hydroxide)	12.30
Group B (Triple Antibiotic Paste)	41.00
GroupC (Turmeric + Calcium hydroxide)	10.70

Table 2 Normality of scores in three groups (Group A, Group B, Group C against Enterococcus faecalis) by Kolmogorov Smirnov test

Materials	Z-value	p-value
Group A	0.7090	0.6970
Group B	0.7550	0.6190
Group C	0.7090	0.6970

Note: The scores in three groups (Group A, Group B, Group C) follows a normal distribution. Therefore, the parametric one way ANOVA followed by Tukeys multiple posthoc procedure test were applied.

Table 3 Summary in three materials

Groups	N	Min	Max	Mean	SD	SE	95% Confidence Interval for Mean	
							Lower Bound	Upper Bound
Group A	10	11.00	14.00	12.30	0.95	0.30	11.62	12.98
Group B	10	39.00	44.00	41.00	1.56	0.49	39.88	42.12
Group C	10	9.00	12.00	10.70	0.95	0.30	10.02	11.38

The above table represents the summary in three materials with 95% confidence intervals

Table 4 Intergroup comparison of zone of inhibition between different groups.

Inter group	Mean difference	Std.error	P-value
A vs B	-28.70	0.53	0.0001*
A vs C	1.60	0.53	0.0150*
B vs C	30.30	0.53	0.0001*

*p<0.05; Signicant

Table 5 Comparison of three materials (Group A, Group B, Group C) by one way ANOVA test.

Sources of variation	Df	Sum of Squares	Mean Square	F-value	p-value
Between Groups	2	5814.47	2907.23	2054.8510	0.0001*
Within Groups	27	38.20	1.42		
Total	29	5852.67			

*p<0.05

The results of the above table, clearly showing that, there is a significant difference between three materials (Group A, Group B, Group C) with respect to mean scores (F=2054.8510, p<0.05) at 5% level. It means that, the mean score is different in three materials (Group A, Group B, Group C).

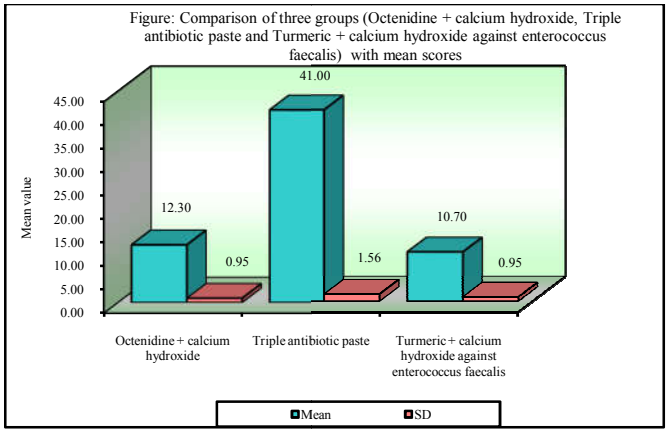
Table 6 Pair wise comparison of three groups (A,B,C) by Tukeys multiple posthoc procedures

Materials		Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	Group B	-28.70	0.53	0.0001*	-30.02	-27.38
	Group C	1.60	0.53	0.0150*	0.28	2.92
Group B	Group C	30.30	0.53	0.0001*	28.98	31.62

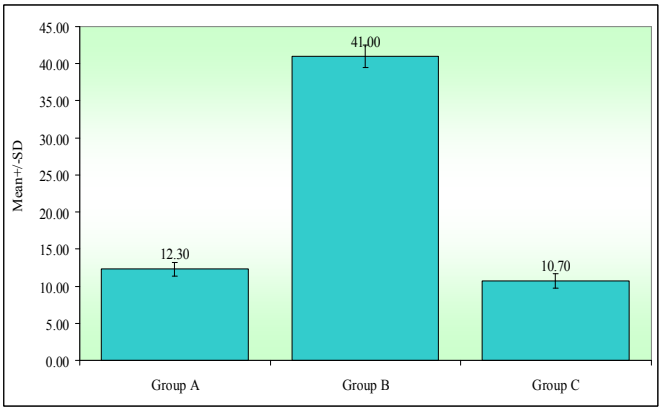
*p<0.05

From the results it can be seen that

1. A significant difference is observed between Group A and Group B with respect to mean scores of the variable at 5% level. It means that, the mean score is significantly higher in Group B as compared to group A.
2. A significant difference is observed between Group A and Group C with respect to mean scores of the variable at 5% level. It means that, the mean score is significantly higher in Group Aas compared to Group C.
3. A significant difference is observed between Group B and Group C against enterococcus faecalis with respect to mean scores of the variable at 5% level. It means that, the mean score is significantly higher in Group B as compared to Group C.



Graph 1 Comparison of three groups (Octenidinedihydrochloride + Calcium Hydroxide, Triple Antibiotic Paste and Turmeric + Calcium Hydroxide against Enterococcus faecalis) with mean scores.



Graph 2 Mean values of Group A, Group B and Group C

DISCUSSION

In many primary root canal infections and treatment failures, mechanical preparation and irrigation cannot eliminate all the bacteria from the infected root canal. In these cases, the use of intracanal medicament isessential to help disinfect the infected root canal system. Calcium hydroxide is commonly used as an intracanal medicament. The present study examined the effects of different intracanalmedicaments against E. faecalis, including Octenidinedihydrochloride, Triple Antibiotic Paste and Turmeric.[2]

E. faecalis was chosen as a test organism because it is a facultative organism that is easy-to-grow, non-fastidious, efficiently and rapidly colonizes tubules (Orstavik&Haapasalo 1990). It has been used extensively in endodontic research because it has been found to be present in 63% of teeth with post-treatment disease (Hancock *et al*. 2001).[9]It plays a major role in persistent periapical diseases.[7]

Calcium hydroxide has been recommended for use as intracanal medication based on its antibacterial, anti-resorptive and tissue-dissolving properties. When used as intracanal medicament, calcium hydroxide has been shown to be effective in eliminating bacteria from the root canal space. Their lethal effects on bacterial cells are probably due to the damage of the bacterial cytoplasmic membrane, denaturation of protein and damage to DNA. Although hydroxyl ions possess antibacterial effects, rather high pH values are required to destroy microorganisms (Venigalla *et al*, 2015).[3]

TheTriple antibiotic paste is a mixture of metronidazole, ciprofloxacin and minocycline which is used as an intracanal medicament for disinfection of immature necrotic teeth, during regenerative procedures. The action of this mixture against various endodontics microbes had been studied extensively and proved to be beneficial.[10]

Adl *et al* in 2012 determined the in vitro antimicrobial ability against Enterococcus faecalis of triple antibiotic paste and its components compared with calcium hydroxide mixtures. They concluded that Triple antibiotic paste with normal saline would be preferred medicament against E. faecalis and amongst it's three component.The study showed that the higher the concentrations (25 µg per mL to 200 µg per mL) of antibiotics or calcium hydroxide, the greater its' antibacterial efficacy.[11]Mozayeni *et al* in 2014 evaluated the antimicrobial activity of four intracanal medicaments on Enterococcus Faecalis. They concluded that Triple antibiotic paste and Chlorhexidine gel showed better antibacterial efficacy than calcium hydroxide and can be used as an alternative intracanal medicaments in root canal therapies.[7]

Makkar *et al* in 2017 evaluated the antibacterial and antifungal properties of Octenidine hydrochloride (0.025%,0.05% and 0.1 %) and Chlorhexidine (0.2%, 1%, 2%) as endodontic root canal irrigant. They concluded that Octenidine hydrochloride is an effective endodontic irrigant. In the present study, 2% Octenidine hydrochloride is used.[8]Dogan *et al* in 2008 compared the short-term relative antibacterial effects of OCT and CHX. Their results showed that, OCT was found favorably more effective than CHX in its antibacterial activity, both in vitro and in vivo.[12]Hegde *et al* in 2013 compared the antimicrobial activity of 2% sodium hypochlorite, propolis, neem leaf extract, turmeric and liquorice against E. Faecalis and C. Albicans. They found that the aqueous extract of turmeric showed mild activity against Enterococcus faecalis.[5]
In the present study, the antibacterial activity of Group B that is Triple Antibiotic Paste (mean value – 41) was better than Group A that is Octenidinedihydrochloride plus Calcium hydroxide (mean value -12.30) and Group C that is Turmeric plus Calcium hydroxide (mean value -10.70) against Enterococcus faecalis.

CONCLUSION

Under the limitation of this study,

- All the materials showed antibacterial activity against Enterococcus faecalis.
- Group B(Triple Antibiotic Paste) showed best efficacy with highest zone of inhibition against E.faecalis and consider as more powerful root canal medicament when compared with that of Group A (Octenidinedihydrochloride +Calcium hydroxide) and Group C(Turmeric+Calcium hydroxide).

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