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ANTIBACTERIAL EFFICACY OF ZINC OXIDE PASTE WITH VARIOUS ESSENTIAL OILS AGAINST *ENTEROCOCCUC FAECALIS* AND ITS COMPARISON WITH ZINC OXIDE EUGENOL PASTE

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ABSTRACT

Aim: To evaluate the susceptibility of *E.faecalis* to zinc oxide paste with various essential oils like tea tree oil, thyme oil, peppermint oil and to compare it with commonly used zinc oxide eugenol paste.
Methodology: Three different test materials i.e; zinc oxide with tea tree oil (ZnO+TT), zinc oxide with thyme oil (ZnO+Th), zinc oxide with peppermint oil (ZnO+P) were freshly mixed. All the test materials were compared with zinc oxide eugenol paste (ZnO+E) which is routinely used as root canal filling material in pediatric dentistry. Culture of *E.faecalis* was uniformly spread on the agar plates. Wells of 6mm diameter were punched in Muller Hincton agar plates into which test materials were added. Procedure was completed by using agar diffusion method. All the plates were kept in incubator at 37ºC for 24 hrs and inhibition zones were measured in mm. Results of test materials were compared with zinc oxide eugenol paste statistically using ANOVA and Tukey's post-hoc test at a significance level of 5%
Results: Zones of inhibition against *E.faecalis* were more for ZnO+Th oil (28.00±1.78) followed by ZnO+TT oil (19.00±4.51); ZnO+E oil (10.83±1.47) and ZnO+P oil (8.00±0.00) with statistically significant differences (0.0001, S,p<0.05)
Conclusion: After comparing ZnO+E oil paste, it can be concluded that ZnO+Th oil paste showed higher zones of inhibition suggestive of it, being the effective antimicrobial paste which can be used as root canal filling paste in primary teeth. ZnO+TT oil also showed good antimicrobial efficacy in comparison to ZnO+E oil paste.

INTRODUCTION

When the pulp of deciduous teeth gets affected either by caries or trauma, pulp therapy has to be performed in such cases so as to maintain the primary teeth in its functional and healthy state until it is replaced by their permanent successors (Garcia-Godoy 1987). Complex anatomy of root canals of primary teeth makes it difficult to achieve cleansing properly by instrumentation and use of irrigating solutions (Carrotte 2005). Numerous materials have been tried as intracanal antimicrobials (Aravind *et al* 2006). Zinc oxide eugenol cement is widely used as root canal filling material for the deciduous teeth (Sweet 1930). There are disadvantages associated with zinc oxide paste like slower rate of resorption than the roots of deciduous teeth (Mortazavi & Mesbahi 2004, Ozalp *et al* 2005). It is also said that it should not be considered as ideal root canal filling material for deciduous teeth as it has limited antimicrobial activity (Tchaou *et al* 1996). *Enterococcus faecalis* are gram-positive cocci which are found either singly or in pairs or in short chains. These are facultative anaerobes which are predominantly seen in cases with root canal failures or persistent root canal infections (Molander *et al* 1998, Sundqvist *et al* 1998, Distel *et al* 2002). Tea tree oil is an essential oil with antimicrobial effect due to number of small terpenoids and phenol compounds (Gustafson *et al* 1998, Mann *et al* 2000, Cox *et al* 2000). Thymol, an active component of Thyme oil appears to inhibit growth of oral pathogens in the mouth and it is said that in combination with other essential oils, it may reduce dental caries (Shapiro & Guggenheim 1995, Yu *et al* 2000). Menthol is the active ingradient in peppermint oil. Other important constituents in peppermint oil are menthyl acetate, 1,8-cineole, limonene,

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beta-pinene, and beta-caryophyllene. This medicinal plant has various properties like analgesic, antiseptic, astringent, anesthetic, carminative, decongestant, expectorant, stimulant; also effective against ulcer and stomach problems and inflammatory diseases (Alankar 2009, Schmidt *et al* 2009).

Search of newer materials with effective antimicrobial properties has led to conduct the present study with use of ZnO+TT paste, ZnO+Th paste and ZnO+P paste to determine antimicrobial efficacy against the most resistant microorganism i.e; *E.faecalis*. These cements were compared with the routinely used zinc oxide eugenol cement.

MATERIALS AND METHODS

Present study had been approved by institutional ethical committee.

Essential oils: Essential oils used in the present study were tea tree oil, thyme oil, peppermint oil (Aromatantra, Mumbai) which were mixed with zinc oxide powder and compared with zinc oxide eugenol paste (Prime Dental Products Pvt. Ltd., Thane).

Powder liquid ratio: For all the test materials and ZOE paste, 1 scoop (0.2gm) of zinc oxide powder was taken and mixed with 7 drops of oil (0.07cc) on the dry and sterile glass slab using cement spatula (Tchaou *et al* 1995).

Test microorganism: Strain of *E.faecalis* used for the present study was obtained from the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India. *Enterococcus faecalis* (ATCC 29212) strain was used for the study.

Agar medium: Muller Hinton Agar was used as growth medium for evaluating the susceptibility of *Enterococcus faecalis.*

Procedure: Strains of *Enterococcus faecalis* from the stock culture available in the microbiology department was taken and added to 5ml BHI broth which was incubated at 37°C for 24 hrs. Sub-culture of *E.faecalis* strains was done on blood agar plates. It was incubated at 37°C for 24 hrs. and microbial colonies were inoculated in nutrient broth. Density of broth was adjusted to 0.5 McFarland opacity standard scale. Procedure was carried out in laminar flow chamber to prevent contamination. Microbial colonies were taken from the nutrient broth and uniformly spread on MH agar. Holes of 6 mm diameter were made at two equal distance points. Fresh mixed test material and control material was then filled in the hole. To achieve pre-diffusion of the test materials in MH-agar Plates, plates were kept for 2 hrs. at room temperature. Experiment was repeated six times for each microorganism. All the plates were kept in incubator at 37ºC for 24 hrs. Diameter of zones of inhibition in millimeters around each test material was measured in with the help of HiAntibiotic Zone Scale (HiMedia). Zones having larger diameters were considered as having antibacterial activity against *E.faecalis*.

Statistical Analysis

Data of antibacterial activity was analyzed statistically using ANOVA and Tukey's post-hoc test at a significance level of 5% using the Graph Pad Prism 4 software.

RESULTS

Table 1 shows zones of bacterial inhibition in mm of ZO+TT oil paste, Zo+Th oil paste, Zo+P oil paste and Zo+E oil paste against *E.faecalis.* Diameters in mm of zone of inhibition for ZO+Th oil paste was largest against *e.faecalis* i.e; 28.00±1.78. Zones of inhibition in mm for ZO+TT oil paste against *e.faecalis* was 19.00±4.51, with ZnO+E oil paste, it was 10.83±1.47 and with ZnO+P oil paste it was 8.00±0.00.

Table 1. Zones of inhibition in mm of ZnO+TT oil, ZnO+Th oil, ZnO+P oil and Zno+E oil against *E.faecalis*

Root canal filling materials	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
ZnO+TT	6	19.00	4.51	1.84	14.26	23.73
ZnO +Th	6	28.00	1.78	0.73	26.12	29.87
ZnO +P	6	8.00	0.00	0.00	8.00	8.00
ZnO +E	6	10.83	1.47	0.60	9.28	12.37

Table 2. Zones of inhibition in mm of ZnO+TT oil, ZnO+Th oil, ZnO+P oil and Zno+E oil against *E.faecalis* by using one way ANOVA

Source of variation	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	1457.12	3	485.70	75.40	0.0001
Within Groups	128.83	20	6.44		S,p<0.05
Total	1585.95	23			

S: significant

Table 3. Zones of inhibition in mm of ZnO+TT oil, ZnO+Th oil, ZnO+P oil and Zno+E oil against *E.faecalis* by using multiple comparison: Tukey Test

Root canal filling materials		Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
ZnO+TT	ZnO +Th	-9.00	1.46	0.0001,S	-13.10	-4.89
	ZnO +P	11.00	1.46	0.0001,S	6.89	15.10
	ZnO +E	8.16*	1.46	0.0001,S	4.06	12.26
ZnO+Th	ZnO +P	20.00*	1.46	0.0001,S	15.89	24.10
	ZnO +E	17.16*	1.46	0.0001,S	13.06	21.26
ZnO+P	ZnO +E	-2.83	1.46	0.246,NS	-6.93	1.26

S: significant, NS: Not significant

Table 2 shows zones of inhibition in mm of ZnO+TT oil, ZnO+Th oil, ZnO+P oil and Zno+E oil between groups and within groups against *E.faecalis* by using one way ANOVA and the difference was found to be statistically significant (p-value: 0.0001, p<0.05). Table 3: Zones of inhibition in mm of ZnO+TT oil, ZnO+Th oil, ZnO+P oil and Zno+E oil against *E.faecalis* by using multiple comparison: Tukey Test. When antimicrobial efficacy of ZnO+TT oil paste was compared with other test materials and ZnO+E oil paste, it was observed that difference with ZnO+Th oil paste, ZnO+P oil paste and ZnO+E oil paste was statistically significant (p-value: 0.0001, p<0.05). When antimicrobial efficacy of ZnO+Th oil paste was compared with ZnO+P oil paste and ZnO+E oil paste, it was observed that difference was statistically significant (p-value: 0.0001, p<0.05). When ZnO+P oil was compared with ZnO+E oil paste to evaluate antimicrobial efficacy against *E.faecalis*, it was observed that difference was not statistically significant (p-value: 0.246, p>0.05).

DISCUSSION

Enterococcus faecalis is the gram positive, facultative anaerobic microorganism found in the persistent perapical infections and failed root canals. (Stuart *et al* 2006). Its presence in deciduous root canals cannot be ignored because of the complex morphology of root canals of deciduous teeth. Roots of deciduous teeth are curved to accommodate the tooth buds of permanent successors. Curved roots have thin, tortuous, curved, ribbon like canals with numerous accessory canals which are connected with each other with lateral interconnecting canals. These canals if get infected, becomes difficult to be cleansed properly by proper instrumentation and irrigation of canals. Action of zinc oxide cement has been said to be because of its eugenol content. Eugenol is also known as allyl guaiacol or eugenic acid. It is the active ingredient of oil of cloves consisting of 70-80% of its bulk. Clove oil is useful in the treatment of dental caries which has been used in dentistry since long time. Zinc oxide eugenol paste has numerous clinical applications including its use as sedative dressing, pulp capping agent and root canal sealer etc. Eugenol is said to be bactericidal at high concentrations of 10^{-2}-10^{-3} Mol/L, but at the same conc. It is toxic to the pulp. At a low concentration, it acts as a local anesthetic. It has analgesic, anti-inflammatory and antibacterial properties also. Even though zinc oxide eugenol is widely used in pediatric dentistry, it has disadvantages associated with it (Jyoti 2005).

In search of plant essential oils which can exhibit the antimicrobial activity against the most resistant microorganism i.e, *e.faecalis,* present study had used zinc oxide powder with various essential oils like tea tree oil, thyme oil, peppermint oil. Verma *et al* (2015) had evaluated the antimicrobial efficacy of 1% or 2% Chlorhexidine (CHX) + calcium hydroxide (CH), Zinc Oxide Eugenol (ZOE) and CH + iodoform (Metapex) in dentinal tubules of deciduous teeth infected with *E. faecalis* by using viability stain and confocal laser scanning microscope. It was concluded from the study that all the materials effectively showed antimicrobial effect against *E. faecalis* in dentine of primary teeth, efficacy increased with longer contact with 1% CHX+ CH. Study of Wang *et al* (2014) had used the novel dentine infection model to evaluate the antimicrobial effects of Endosequence BC sealer (BC sealer), AH Plus and pulp canal sealer EWT (PCEWT) against *E. faecalis* biofilms in dentinal tubules. Root canal sealers were placed on the root canal wall of the dentin specimens which were kept in humid conditions at 37°C for 1, 7, and 30 days. Dead and live bacteria in the dentinal tubules were evaluated by confocal laser scanning microscopy. It was seen that BC sealer and AH Plus had shown superior antibacterial effects than PCEWT Queiroz AM et al, 2009[23] had evaluated the antibacterial activity of zinc oxide eugenol cement (ZOE), Sealapex sealer, Calen paste thickened with zinc oxide (Calen/ZO) and EndoREZ sealer against *E. faecalis, K. rhizophila, S.mutans, S. aureus and E.coli* using the agar diffusion test. Calen/ZO had shown highest antibacterial activity against E. faecalis. Calen paste and Calen/ZO showed larger zones of inhibition against *E.faecalis* when compared with 1% CHX. Study had concluded that antibacterial activity of root canal filling materials for primary teeth against microorganisms commonly seen in endodontic infections in decreasing order were: ZOE> Calen/ZO> Sealapex> EndoREZ. Smadi *et al* (2009) had conducted study to evaluate the antimicrobial activity of 3 zinc oxide-based sealers, 4 resin-based sealers and 2 calcium hydroxide-based sealers against 2 strains of *S. aureus*, 2 strains of *C. albicans* and 1 strain of *E. faecalis*. The antimicrobial activity of root canal sealers observed in descending order was: Sealite Regular> Cortisemol Dentalis KEZ> AH26> Sealapex> Acroseal/Topseal> Endorez/AH plus. Root canal sealers having formaldehyde and eugenol were found to be effective against the microorganisms.

In the in vitro study carried out by Hoelscher *et al* (2006), antimicrobial effects of antibiotics like amoxicillin, penicillin, clindamycin, metronidazole, and doxycycline added to Kerr Pulp Canal Sealer EWT were evaluated against *E. faecalis*. Results of the study showed that the sealer and antibiotic combination containing amoxicillin, clindamycin, penicillin and doxycycline had a significant difference in the zones of inhibition as compared to Kerr EWT sealer alone. In the present study, susceptibility of *E.faecalis* to ZnO+TT oil paste, ZnO+Th oil paste, ZnO+P oil paste in the form of zone of inhibition was evaluated and compared with routinely used ZnO+E oil paste. It was surprising to observe that diameters of zones of inhibition of ZnO+Th oil paste were largest (28.00±1.78) against *E.faecalis* when compared with Zno+E oil paste (10.83±1.47). Zones of inhibition of ZnO+TT oil paste were also larger (19.00±4.51) when compared to Zno+E oil paste (10.83±1.47). But zones of inhibition of Zno+E oil paste (10.83±1.47) were larger as compared to ZnO+P oil paste (8.00±0.00). Diameters of zones of inhibition against *E.faecalis* in decreasing order were ZnO+Th oil paste (28.00±1.78), followed by ZnO+TT oil paste (19.00±4.51), Zno+E oil paste (10.83±1.47) and ZnO+P oil paste (8.00±0.00) respectively. But the difference was significant statistically (0.0001, S,p<0.05). In the present study depending upon the results, it can be said that ZnO+Th oil paste, ZnO+TT oil paste can be effectively used as root canal filling material against *E.faecalis* in primary teeth. Order of preference in descending order for the use of these root canal filling materials against the microorganism, *E.faecalis* for primary teeth can be: ZnO+Th oil paste> ZnO+TT oil paste> ZnO+E oil paste> ZnO+P oil paste.

Conclusion

It can be concluded from the present study that ZnO+Th oil paste, ZnO+TT oil paste can be effectively used as root canal filling material against *E.faecalis* in primary teeth. ZnO+E paste showed better antimicrobial efficacy than ZnO+P oil paste.

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