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RESEARCH ARTICLE

EVALUATION OF *IN VITRO* ANTIBACTERIAL EFFECT OF CALCIUM HYDROXIDE PASTE WITH TEA TREE OIL, THYME OIL AND PEPPERMINT OIL AGAINST *ENTEROCOCCUS FAECALIS* AND ITS COMPARISON WITH THAT OF CALCIUM HYDROXIDE SALINE PASTE IN PRIMARY TEETH

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ABSTRACT

Background: Various essential oils like tea tree oil, thyme oil, peppermint oil were mixed with calcium hydroxide cement to form the paste. Susceptibility of *Enterococcus faecalis* with all test materials was evaluated which was then compared with calcium hydroxide saline paste.
Materials and Methods: Different types of pastes used in the study were: calcium hydroxide with tea tree oil paste (CaOH+TT), calcium hydroxide with thyme oil paste (CaOH+Th), calcium hydroxide with peppermint oil paste (CaOH+P), calcium hydroxide with saline paste (CaOH+S). CaOH+S was used as control paste with which all the other test materials were compared. Susceptibility of *E.faecalis* with all the test materials and control material was evaluated in terms of zones of inhibition using agar diffusion method. In this method, wells of 6mm diameter were punched in Muller Hincton agar, filled with test and control material, incubated at 37ºC for 24 hrs. and zones of inhibition measured in mm. All the data was statistically analyzed using ANOVA and Tukey's post-hoc test at the significance level of 5%
Results: It was observed that susceptibility of *E.faecalis* in the form if zones of inhibition (in mm) in decreasing order were: CaOH+Th paste (33.33±1.03)> CaOH+TT paste (24.00±1.78)> CaOH+S paste (17.33±3.01)> CaOH+P paste (12.66±1.03) with statistically significant difference between and within groups (0.0001, $p<0.05$).
Conclusion: Best results were obtained with CaOH+Th paste followed by CaOH+TT paste, CaOH+S paste and CaOH+P paste. CaOH+Th paste can be effectively used as root canal filling material in primary teeth against the most resistant microorganism i.e; *enterococcus faecalis.*

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INTRODUCTION

Root canal infections are usually polymicrobial in nature. When pulp of primary teeth is affected due to caries, pulp therapy is performed to remove the infected pulp so as to keep the primary teeth in functional state. When root canals of primary teeth are involved, complex anatomy of root canals of primary teeth consisting of various accessory canals, interconnecting lateral canals make it impossible to completely remove the infected pulp. Calcium hydroxide has benefit in the field of dentistry as an intracanal medicament due to its bactericidal effect because of its high pH approximately 12.5. It is considered that most of the microorganisms involved in endodontic infections are killed in such a highly alkaline environment provided by calcium hydroxide (Stanley *et al.*, 1966; Massler, 1967; Dhull *et al*., 2013). *Enterococcus faecalis* is Gram positive cocci that occur singly in pairs or in short chains (Hedge, 2009). It is a facultative anaerobe present in small proportion of the flora of untreated canal and is a predominant bacteria implicated in root canal failures and

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persistent root canal infections (Molander *et al.,* 1998; Sundqvist *et al.,* 1998; Distel *et al.,* 2002). Antimicrobial effect of Tea tree oil, an essential oil is due to small terpenoids and phenol compounds (Gustafson *et al.,* 1998, Cox *et al.,* 2000, Mann *et al.,* 2000). Thyme oil inhibits growth of oral pathogens in the mouth due to the presence of thymol (Shapiro and Guggenheim, 1995; Yu *et al.,* 2000). Peppermint oil shows its antimicrobial efficacy due to its active ingredient i.e; menthol. Along with antimicrobial efficacy, these medicinal essential oils have been proved to show various effects like analgesic, antiseptic, astringent, anesthetic, carminative, decongestant, expectorant and also effective against inflammatory diseases. Considering the beneficial effects of these essential oils and also the known bactericidal properties of calcium hydroxide, the present study was carried out to evaluate the antibacterial effect of calcium hydroxide paste with tea tree oil, thyme oil and peppermint oil against *enterococcus faecalis* and its comparison with that of calcium hydroxide saline paste as a root canal sealer in primary teeth.

MATRIALS AND METHODS

Institutional ethical committee was obtained before starting the study. Essential oils like tea tree oil, thyme oil, peppermint oil were procured from Aromatantra, Mumbai. All these oils were mixed with calcium hydroxide powder (Prime Dental Products Pvt. Ltd., Thane) to form the test materials: CaOH+TT paste, CaOH+Th paste, CaOH+P paste which were compared with the control material i.e; Calcium hydroxide mixed with saline (CaOH+S paste). Powder liquid ratio was standardized by mixing 0.2gm of calcium hydroxide powder with 0.07cc of oil/ saline on sterile dry glass slab using the sterile cement spatula (Tchaou *et al.,* 1995).

Strain of *Enterococcus faecalis* (ATCC 29212) which was obtained from Microbiologics, USA, Hi Media, Pvt., Ltd., was procured from the department of Microbiology, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India. Agar medium used for the study was Muller Hinton Agar. 5ml BHI broth containing strain of *E.faecalis* was incubated at 37°C for 24 hrs. and sub-cultured on blood agar plates; again incubated at 37°C for 24 hrs. Microbial colonies were inoculated in nutrient broth. Density of nutrient broth was adjusted to 0.5 McFarland scale. Procedure was carried out in Biosafety Cabinet II. Microbial colonies taken from the nutrient broth were uniformly spread on MH agar plates. Holes having 6 mm diameter were punched at two equidistatnt points. Test materials and control material was filled in the punched holes. All the plates were kept at room temperature to allow for pre-diffusion of material and after that kept in incubator at 37ºC for 24 hrs. All the experiment was repeated 6 times. Zones of inhibition in millimeters around each test material and control material was measured with the help of HiAntibiotic Zone Scale (HiMedia). Zones with larger diameters were considered as having susceptibility of *E.faecalis* to test material or control material. Data of antibacterial activity in the form of zones of inhibition was analyzed statistically using ANOVA and Tukey's post-hoc test at a significance level of 5% using SPSS 17.0 software and the Graph Pad Prism 5.0 version.

RESULTS

Table 1, Table 2, Table 3 and Graph 1 show susceptibility of *Enterococcus faecalis* in the form of zones of inhibition (in mm) to CaOH+TT, CaOH+Th, CaOH+P, CaOH+S paste CaOH+Th paste had shown highest zones of bacterial growth inhibition in mm against *E.faecalis* i.e; 33.33±1.03.

Table 1. Susceptibility of *Enterococcus faecalis* in the form of zones of inhibition (in mm) to CaOH+TT, CaOH+Th, CaOH+P, CaOH+S paste

Material	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CaOH+TT	6	24.00	1.78	0.73	22.12	25.87	22.00	26.00
CaOH+Th	6	33.33	1.03	0.42	32.24	34.41	32.00	34.00
CaOH+P	6	12.66	1.03	0.42	11.58	13.75	12.00	14.00
CaOH+S	6	17.33	3.01	1.22	14.17	20.49	14.00	20.00

Table 2. One Way ANOVA showing susceptibility of *Enterococcus faecalis* in the form of zones of inhibition (in mm) to CaOH+TT, CaOH+Th, CaOH+P, CaOH+S paste

Source of variation	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	1447.33	3	482.44	134.01	0.0001
Within Groups	72.00	20	3.60		S, p<0.05
Total	1519.33	23			

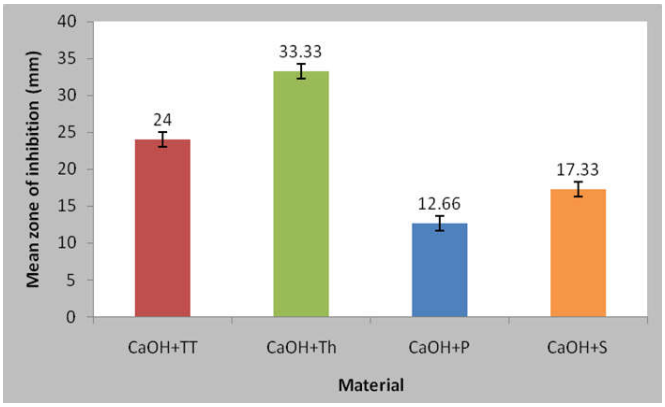
S: Significant

Table 3. Multiple Comparison: Tukey Test showing susceptibility of *Enterococcus faecalis* in the form of zones of inhibition (in mm) to CaOH+TT, CaOH+Th, CaOH+P, CaOH+S paste

Material		Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
CaOH+TT	CaOH+Th	-9.33	1.09	0.0001,S	-12.39	-6.26
	CaOH+P	11.33	1.09	0.0001,S	8.26	14.39
	CaOH+S	6.66	1.09	0.0001,S	3.60	9.73
CaOH+Th	CaOH+P	20.66	1.09	0.0001,S	17.60	23.73
	CaOH+S	16.00	1.09	0.0001,S	12.93	19.06
CaOH+P	CaOH+S	-4.66	1.09	0.002, S	-7.73	-1.60

S: Significant

Zones of inhibition in mm shown by other materials in decreasing order were CaOH+TT paste (24.00±1.78), CaOH+S paste (17.33±3.01) and CaOH+P paste (12.66±1.03) (Table 1). Difference between and within groups was found to be statistically significant (0.0001, $p<0.05$) (Table 2). Table 3 shows the differences in zones of inhibition in mm amongst various materials against *E.faecalis*. Difference related to zones of bacterial growth inhibition against *E.faecalis* between CaOH+TT vs CaOH+Th (0.0001, $p<0.05$), CaOH+TT vs CaOH+P (0.0001, $p<0.05$), CaOH+TT vs CaOH+S (0.0001, $p<0.05$), CaOH+Th vs CaOH+P (0.0001, $p<0.05$), CaOH+Th vs CaOH+S (0.0001, $p<0.05$) and CaOH+P vs CaOH+S (0.002, $p<0.05$) was found to be significant statistically.



Graph 1. Susceptibility of *Enterococcus faecalis* in the form of zones of inhibition (in mm) to CaOH+TT, CaOH+Th, CaOH+P, CaOH+S paste

DISCUSSION

Anatomic variation in the root canal system of primary teeth makes it difficult to completely eliminate the infection from the root canals. Various root canal medicaments are available in Pediatric Dentistry for removal of the microorganisms associated with infected root canals of primary teeth. Calcium Hydroxide was introduced in endodontics by Hermann in 1920 (Hermann, 1920). Calcium hydroxide is an antibacterial agent due to its high pH. Its high pH influences the specific activity of the proteins of the membrane and can lead to alterations in the ionization state of organic components. Thus it denatures bacterial endotoxin and causes bactericidal effect. *Enterococcus faecalis* has potential to colonize dentinal tubules, isthmus, rami, lateral and accessory canals. It is found in canals of root canal treated teeth with periapical lesions. It is said that root canal hardly contains the structural components having nutrient rich medium. But it is observed that *enterococcus faecalis* may survive on serum components from the dentinal fluid (Molander *et al.,* 1998; Sundqvist *et al.,* 1998; Distel *et al.,* 2002; Mistry *et al.,* 2014). As suggested by Portenier *et al*. (2005), *E. faecalis* is very resistant to calcium hydroxide because of two reasons: 1) the "proton pump" which lowers internal pH of the cell thereby maintaining the pH haemostasis. 2) *E. faecalis* is unable to survive at pH 11.5, but dentin exhibits inhibitory effect thereby buffering the pH. In the study of Verma *et al.* (2015), inclusion of 1% chlorehexidine in calcium hydroxide improved its antimicrobial efficacy. Antimicrobial efficacy of two antibacterial and two obturating pastes was evaluated in dentinal tubules of primary teeth infected with *Enterococcus faecalis* using viability stain and confocal laser scanning microscope (CLSM). Antibacterial pastes used in the study were: 1% or 2% Chlorhexidine (CHX)+calcium hydroxide (CH), Zinc Oxide Eugenol (ZOE) and CH+iodoform (Metapex). Dentinal tubules were infected with *E. faecalis* by centrifugation of the suspension of bacteria. Two samples were subjected to antibacterial pastes exposure after 1, 7 and 15 days. It was observed in the study that antimicrobial efficacy of paste in decreasing order was: 1%CHX+CH (15 days)> ZOE (15 days)> Metapex (15 days)> 2%CHX+CH (15 days)> 2%CHX+CH (7 days)> 2%CHX+CH (1day)> 1%CHX+CH (7 days)> 2%CHX+CH (15 days)> Metapex (1 day)> ZOE (1 day)> ZOE (7 days). All materials were effective against *E. faecalis* in dentine of primary teeth and their effect was found to increase with longer contact with 1%CHX+CH at day 15. Antimicrobial effects of antibiotics like amoxicillin, clindamycin, penicillin, doxycycline and metronidazole added to Kerr Pulp Canal Sealer EWT were evaluated against *E. faecalis* in the study of Hoelscher *et al* (2006). From the results of the study, it was observed that the sealer with antibiotic combinations with amoxicillin, clindamycin, penicillin and doxycycline had a significant difference in their zones of inhibition in comparison to Kerr EWT sealer alone.

Very few studies are available in the literature showing the use of calcium hydroxide with essential oils. As found in literature when calcium hydroxide is mixed with either distilled water or saline, it dissociates faster and its availability of Ca^+ and OH^- ions will be only for short period of time. Therefore the purpose of using tea tree oil, thyme oil, peppermint oil with calcium hydroxide powder in the present study was that if calcium hydroxide powder will be mixed with the essential oils used in the study, will make Ca^+ and OH^- ions available for longer period of time for its action at targeted area. In the present study, zones of inhibition (in mm) obtained in decreasing order were: CaOH+Th paste (33.33±1.03)> CaOH+TT paste (24.00±1.78)> CaOH+S paste (17.33±3.01)> CaOH+P paste (12.66±1.03). *E.faecalis* had shown its susceptibility more to CaOH+Th paste as compared the routinely used calcium hydroxide powder with saline (CaOh+S) paste. Difference between and within groups was statistically significant (0.0001, $p<0.05$).

Conclusion

Depending upon the results of the study, zones of inhibition obtained in case of CaOH+Th paste were larger showing good antibacterial effect as compared to other test materials and control paste. Therefore it can be used as root canal filling material in primary teeth against the most resistant root canal pathogen i,e *enterococcus faecalis.*

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