



A COMPARATIVE STUDY OF AN *IN VITRO* EVALUATION OF ANTIMICROBIAL EFFICACY OF CALCIUM HYDROXIDE MIXED WITH TEA TREE OIL AND CALCIUM HYDROXIDE MIXED WITH SALINE AS ROOT CANAL FILLING MATERIALS IN PRIMARY MOLARS

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ABSTRACT

Present study was aimed to compare *in vitro* antimicrobial activity of calcium hydroxide cement mixed with tea tree oil paste (CaOH+TT) with that of calcium hydroxide mixed with saline paste (CaOH+S) against the microorganisms of root canal infections of primary teeth i.e; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. It was an *in vitro* experimental and microbiological study conducted from March 2016 to April 2016. Present microbiological study was conducted on Muller Hinton agar plates using agar diffusion test. CaOH+TT oil paste and CaOH+S paste were freshly mixed and placed in the wells of MH agar plates and incubated at 37°C for 24 hrs. After 24 hrs., zones of inhibition around the wells showing antimicrobial efficacy of test materials were measured in millimeters. Statistical analysis was done by using descriptive and inferential statistics using One way ANOVA and Multiple comparison: Tukey test. P value <0.05 was considered as level of significance. CaOH+TT oil paste showed larger zones of growth inhibition against *E. Coli* followed by *E. faecalis*, *P.aeruginosa* and *E. faecalis* respectively. In case of CaOH+S paste, growth inhibitory zones in decreasing order against microorganisms were *E. Coli* > *Staph. Aureus* = *E. faecalis* > *P. aeruginosa*. Difference was found to be statistically significant in CaOH+TT oil paste (p-value: 0.0001, p<0.05) and not significant in CaOH +S paste (0.373, p>0.05). In the present study, as the bacterial growth inhibitory zones were wider in CaOH +TT oil paste as compared to CaOH+S paste against all the microorganisms, CaOH +TT oil paste can be used as a root canal filling paste in primary teeth effectively.

KEYWORDS: CaOH +Tea Tree oil paste, CaOH +Saline paste, antimicrobial efficacy.

INTRODUCTION

Root canal infections of primary teeth are polymicrobial in nature (Edwards S and Nord CE, 1972; Tomi -Karovi K and Jelinek E, 1971). The success of endodontic treatments depends upon the effectiveness of antimicrobial agents to eliminate the living microorganisms from the infected root canals.³ Primary teeth, due to its complex anatomy need to be properly biomechanically prepared and thoroughly irrigated. Several medicaments have been tried in dentistry (Goerig and Camp, 1983; Tchaou *et al.*, 1995; Pabla *et al.*, 1997; Chawla *et al.*, 2001; Amorim L de FG de *et al.*, 2006; Reddy and Ramakrishna, 2007).

Calcium hydroxide has high pH i.e; 12.5-12.8. Its action is due to release of calcium and hydroxyl ions on vital structure of tooth causing hard tissue formation and antimicrobial effect (Spångberg and Haapasalo, 2002). Various combinations of calcium hydroxide have been tried in Pediatric Dentistry such as: calcium hydroxide with sterile water (Tchaou *et al.*, 1995; Chawla *et al.*,

2001; Chawla *et al.*, 2008) with camphorated parachlorophenol, iodoform based paste (Tchaou *et al.*, 1995; eg; Metapex, Vitapex (Pabla *et al.*, 1997; Reddy and Ramakrishna, 2007), iodoform paste (Pabla *et al.*, 1997) eg; Maisto's paste (Pabla *et al.*, 1997), Guedes-Pinto paste (Amorim L de FG de *et al.*, 2006), Endoflas (Fuks and Eidelman, 1991) and zinc oxide and calcium hydroxide with and without sodium fluoride (Chawla *et al.*, 2001; Chawla *et al.*, 2008).

It is said that the vehicle which is used with Ca(OH)₂ to prepare paste influences the velocity and concentration and release of calcium and hydroxyl ions. Vehicle can be either in aqueous or oil form. Aqueous vehicle is either water or saline. When Ca(OH)₂ is mixed with saline/water, it cause rapid release of Ca⁺² and OH⁻. Water/saline has been said to have a high degree of solubility and resorption (Estrela and Pesce, 1996).

Search of oily vehicles have been thought of using tea tree oil in the present study. Tea tree oil is an essential oil with

antimicrobial effect due to number of small terpenoids and phenol compounds. Antimicrobial activity of Tea Tree oil is due to terpinen-4-ol, - terpinol and 1.8-cineole which cause leakage of 260-nm-light absorbing material and render cell susceptible to sodium chloride. Terpinen-4-ol enters microorganism cell membrane and acts against its structural permeability. In this way Tea tree oil can affect the metabolism of certain microorganisms with bactericidal and fungicidal effect (Gustavson *et al.*, 1998; Cox *et al.*, 2000; Mann *et al.*, 2000; Thosar *et al.*, 2016). In the present study, antimicrobial effect of calcium hydroxide powder mixed with tea tree oil was compared with the calcium hydroxide powder mixed with saline.

MATERIALS & METHODS

Present study was approved by institutional ethical committee. In the present study, calcium hydroxide powder was mixed with Tea tree oil (Aromatantra, Mumbai). For comparison, calcium hydroxide powder (Prevest Denpro Limited, Jammu, India) mixed with saline was used. Calcium hydroxide powder used was 1 scoop equivalent to 0.2 g and tea tree oil as well as saline used in the study i.e; liquid used was 7 drops equivalent to 0.07 cc. To get the exact amount of powder and liquid, an electronic balance and micropipette was used. Powder was mixed with liquid on a dry and sterile glass slab using cement spatula at room temperature. Strains of microorganisms used for the study were *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212) and *Pseudomonas Aeruginosa* (ATCC 27853). All the microbial strains used in the study were obtained from the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India. Stock cultures of bacteria were used for the study. Media used for the study were Brain Heart Infusion Broth, Mueller Hinton Agar, blood Agar.

For cultivation of microorganisms, Brain Heart Infusion Broth was used. Blood Agar was used for the growth of microorganisms. Susceptibility of microorganisms was tested by using Mueller Hinton Agar. Stock cultures of microbial strains were added to 5ml BHI broth and incubated at 37°C for 24 hrs. Subculturing was done on blood agar plate. Colonies of microorganisms were then inoculated in nutrient broth for 4-6 hours, turbidity of which was adjusted to 0.5 standard of McFarland opacity standard scale. Complete procedure was performed in laminar air flow chamber. Ninety millimeter diameter petridishes with four millimeter thick Mueller-Hinton agar were used for the bacterial strains. Lawn technique was employed in which bacterial dilutions were swabbed evenly onto agar plates. With the help of open end of 6mm diameter micropipette, punching was done in the agar plates, into which freshly mixed paste was filled. Six time repetitions of the test were done. Muller Hinton agar plates were incubated at 37°C for 24 hrs. Growth inhibitory zones around the paste were measured in millimeters using HiAntibiotic Zone Scale (HiMedia). Zones with wider diameters were interpreted as having greater antimicrobial activity. Statistical analysis was done by using descriptive and inferential statistics using One way ANOVA and multiple comparison: Tukey test. Software used in the analysis was SPSS17.0 version and p<0.05 was considered as level of significance.

RESULTS

Table 1 shows zones of bacterial growth inhibition in mm of CaOH+TT oil paste against four bacterial strains. Zones of bacterial growth inhibition in mm of CaOH+TT oil paste for *Staph. aureus* was 23.33±2.06, for *E. coli* was 26.66±1.03, for *E. faecalis* was 24.00±1.78 and for *P. aeruginosa* was 23.66 ±1.36 respectively.

TABLE 1A: Zones of bacterial growth inhibition in mm of CaOH+TT oil paste against four bacterial strains

Material	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
<i>Staph. Aureus</i>	6	23.33	2.06	0.84	21.16	25.50	22.00	26.00
<i>E.Coli</i>	6	26.66	1.03	0.42	25.58	27.75	26.00	28.00
<i>E.faecalis</i>	6	24.00	1.78	0.73	22.12	25.87	22.00	26.00
<i>P.Aeruginosa</i>	6	23.66	1.36	0.55	22.23	25.10	22.00	25.00

TABLE 1b shows one way analysis of variance of zones of bacterial growth inhibition in mm of CaOH+TT oil paste against four bacterial strains between and within groups with statistically significant difference (0.0001, p<0.05).

TABLE 1b: Zones of bacterial growth inhibition in mm of CaOH+TT oil paste against four bacterial strains using One Way ANOVA

Source of variation	df	Mean Square	F	p-value
Between Groups	4	622.46	277.88	0.0001
Within Groups	25	2.24		S, p<0.05
Total	29	-		

S: Significant

Table 1c shows Multiple Comparison: Tukey Test of zones of bacterial growth inhibition of CaOH+TT oil against four bacterial strains. Difference was statistically significant between *Staph. aureus* and *E. coli* (p-value:

0.006, p<0.05), between *E. coli* and *E. faecalis* (p-value: 0.0036, p<0.05) and between *E. coli* and *P. aeruginosa* (p-value: 0.015, p<0.05). Difference between *Staph.aureus* and *E. faecalis* (p-value: 0.936, p>0.05), between *Staph.*

aureus and *P. aeruginosa* (p-value: 0.995, p>0.05) and between *E. faecalis* and *P. aeruginosa* (p-value: 0.995, p>0.05) was not significant statistically.

TABLE 1c: Zones of bacterial growth inhibition in mm of CaOH+TT oil paste against four bacterial strains using multiple comparison: Tukey Test

Microorganisms		Mean Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
<i>Staph.</i>	<i>E.Coli</i>	-3.33	0.86	0.006, S	-5.87	-0.79
<i>Aureus</i>	<i>E.faecalis</i>	-0.66	0.86	0.936, NS	-3.20	1.87
	<i>P.Aeruginosa</i>	-0.33	0.86	0.995, NS	-2.87	2.20
<i>E.Coli</i>	<i>E.faecalis</i>	2.66	0.86	0.036, S	0.12	5.20
	<i>P.Aeruginosa</i>	3.00	0.86	0.015, S	0.46	5.53
<i>E.faecalis</i>	<i>P.Aeruginosa</i>	0.33	0.86	0.995, NS	-2.20	2.87

S: Significant, NS: Not Significant

Table 2a depicts zones of bacterial growth inhibition in mm of CaOH+S paste against four bacterial strains. Zones of bacterial growth inhibition of CaOH+S paste for *Staph.*

aureus were 17.33±3.26, for *E. coli* was 19.33±1.63, for *E. faecalis* was 17.33±3.01 and for *P. aeruginosa* was 17.00±3.52 respectively.

TABLE 2a: Zones of bacterial growth inhibition in mm of CaOH+S paste against four bacterial strains

Microorganisms	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
<i>Staph Aureus</i>	6	17.33	3.26	1.33	13.90	20.76	12.00	20.00
<i>E.Coli</i>	6	19.33	1.63	0.66	17.61	21.04	18.00	22.00
<i>E.faecalis</i>	6	17.33	3.01	1.22	14.17	20.49	14.00	20.00
<i>P.Aeruginosa</i>	6	17.00	3.52	1.43	13.30	20.69	12.00	20.00

Table 2b shows zones of bacterial growth inhibition of CaOH+S paste by using one way analysis of variance.

Difference was not statistically significant (0.373, p>0.05) between and within groups.

TABLE 2b: Zones of bacterial growth inhibition in mm of CaOH+S paste against four bacterial strains using One Way ANOVA

Source of variation	df	Mean Square	F	p-value
Between Groups	4	8.13	1.11	0.373
Within Groups	25	7.31		NS , p>0.05
Total	29	-		

NS: Not Significant

Table 2c depicts multiple comparisons: Tukey Test of zones of bacterial growth inhibition of CaOH+S paste against four bacterial strains. Difference was not significant statistically between *Staph. aureus* and *E.coli* (p-value: 0.705, p>0.05), between *Staph. aureus* and *E. faecalis* (p-value: 1.000, p>0.05), between *Staph. aureus*

and *P. aeruginosa* (p-value: 1.000, p>0.05), between *E.coli* and *E. faecalis* (p-value: 0.705, p>0.05), between *E.coli* and *P. aeruginosa* (p-value: 0.575, p>0.05), between *E. faecalis* and *P. aeruginosa* (p-value: 1.000, p>0.05).

TABLE 2c: Zones of bacterial growth inhibition in mm of CaOH+S paste against four bacterial strains using multiple comparison: Tukey Test

Microorganisms		Mean Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
<i>Staph. Aureus</i>	<i>E.Coli</i>	-2.00	1.56	0.705, NS	-6.58	2.58
	<i>E.faecalis</i>	0.00	1.56	1.000, NS	-4.58	4.58
	<i>P.Aeruginosa</i>	0.33	1.56	1.000, NS	-4.25	4.91
<i>E.Coli</i>	<i>E.faecalis</i>	2.00	1.56	0.705, NS	-2.58	6.58
	<i>P.Aeruginosa</i>	2.33	1.56	0.575, NS	-2.25	6.91
<i>E.faecalis</i>	<i>P.Aeruginosa</i>	0.33	1.56	1.000, NS	-4.25	4.91

NS: Not Significant

DISCUSSION

As the root canal anatomy of primary teeth is complex with lots of accessory and lateral canals, use of antibacterial root canal filling paste which will remain in root canal for longer time to have prolonged action is necessary. Calcium hydroxide was introduced in

endodontics as a pulp capping agent by Hermann (1920). Calcium hydroxide powder which has formula Ca(OH)₂, is a white odorless powder. Its molecular weight is 74.08. Its solubility in water is low. It is also said that its solubility further decreases when there is rise in temperature rises

(Farhad and Mohammadi, 2005). Dissociation coefficient of calcium hydroxide is 0.17 which makes it release Ca^+ and OH^- in controlled manner (Spångberg and Haapasalo, 2002). Type of vehicle can affect the speed of dissociation of Ca^{+2} and OH^- ions and consequently the antimicrobial properties. As suggested by Kawakami *et al.* (1987), high molecular weight of oily vehicles prolongs the action of the $\text{Ca}(\text{OH})_2$ paste, and Ca^{+2} and OH^- ions are released at lower rate. Paste also remains for a longer time than paste containing aqueous vehicle. Dissociation of $\text{Ca}(\text{OH})_2$ is essential to produce favorable biological and antimicrobial action by release of both calcium and hydroxyl ions (Duarte MAH *et al.*, 2007). It causes calcium hydroxide ions to react with carbonic gas and remove the source of respiration of anaerobic bacteria, while hydroxyl ions maintain a high alkaline environment which is unfavorable for the survival of bacteria. As *Enterococcus Faecalis* is resistant microorganism in root canals, surviving at pH 11.5, also gets killed at pH 12.5. Therefore high pH value is necessary to achieve antibacterial effect (de Andrade Ferreira *et al.*, 2004). Keeping in mind the advantages associated with calcium hydroxide and proven antibacterial effect of tea tree oil, in the present study antimicrobial effect of calcium hydroxide powder mixed with tea tree oil was compared with calcium hydroxide powder mixed with saline against the root canal pathogens of primary teeth i.e; *Staphylococcus Aureus*, *Escherischia Coli*, *Enterococcus faecalis* and *Pseudomonas Aeruginosa*. Results obtained related to antimicrobial effect by using CaOH+TT oil paste were favorable as compared to CaOH+S paste. Antimicrobial effect of CaOH+TT oil paste was highest against *E.coli* followed in decreasing order by against *E. faecalis*, *P. aeruginosa* and *Staph. aureus*. In case of CaOH +S paste, antimicrobial effect was more against *E.coli*; equal effect against *E.faecalis* and *Staph.aureus*; least effect against *P. aeruginosa* was obtained. Reason for getting good antimicrobial effect in CaOH+TT oil paste is because of the oily vehicle i.e; tea tree oil used in the present study which made it available for its prolonged antimicrobial action as compared with that of CaOH+S paste. In CaOH+S paste, vehicle used was saline in which calcium hydroxide powder dissociated at faster rate into calcium and hydroxyl ions, which were available in place only for shorter duration of action. Therefore use of CaOH+TT oil paste will be a better option for endodontic procedures to be carried out in primary teeth to get enhanced antibacterial action.

CONCLUSION

It can be concluded from the present study that as the new material CaOH+TT oil paste consisting of calcium hydroxide powder with tea tree oil which is a plant essential oil with no toxic effects, has shown antimicrobial activity against the four microbial strains of root canal pathogens of primary teeth effectively as compared to CaOH+S paste, it can be used for root canal filling of primary teeth.

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