

A COMPARATIVE STUDY TO EVALUATE THE ANTIBACTERIAL EFFICACY OF VARIOUS INTRACANAL MEDICAMENTS OVER ANAEROBIC MICROORGANISMS IN INFECTED ROOT CANALS OF PRIMARY TEETH.

Dental Science

Satyam Dutt	(MDS, Pedodontist, PRIVATE PRACTITIONER)
Keerti Chandail*	(MDS, Pedodontist, Senior Lecturer, ITS MURADNAGAR) *Corresponding Author
Mehak Kaul	(PGY1 Deptt of oral and maxillofacial pathology, Ohio State University, Columbus, Ohio),
Punit Singh	(BDS, General Dentist)

ABSTRACT

Introduction: The purpose of this study was to determine the antibacterial efficacy of intracanal medicaments which were Calcium hydroxide, 2% Chlorhexidine gluconate, 3 Mix paste and Aloe vera against the anaerobic microorganisms isolated from the infected root canals of primary teeth.

Materials and Methods: An agar diffusion assay method was used to determine the antibacterial efficacy of experimental intracanal medicaments against anaerobic microorganisms isolated from the infected root canals of primary teeth. Medicaments were divided into four groups: Calcium Hydroxide mixed with glycerin, 2% Chlorhexidine gluconate, 3 Mix paste mixed with glycerin and Aloe vera plant extract. A total number of 120 microbial specimens were collected from primary molar teeth with atleast one necrotic canal, abscess or sinus tract using sterile absorbent paper points. The diameters of the growth inhibition zones for each group were recorded and compared using agar diffusin assay.

Results: 3 mix paste was found to be most effective against Clostridium and Streptococci species where as Aloe vera was most effective against Lactobacillus species. E. faecalis showed reduced growth against chlorhexidine gluconate and calcium hydroxide was found to be least effective amongst all the four intracanal medicaments.

Conclusion: The results suggest that the triple antibiotic paste is most effective against mostly all of the microorganisms and aloe vera was effective against Lactobacillus species which is usually resistant to other intracanal medicaments.

KEYWORDS

Antibacterial Efficacy, Calcium Hydroxide, Chlorhexidine Gluconate, Triple Antibiotic Paste, Aloe vera.

Introduction:

Successful Endodontics virtually is a *bacteriologic* game. Final quarter of nineteenth century was arguably the golden age of medical microbiology. The ground breaking work of Louis Pasteur, Robert Koch and others led to the development of broth and agar media that were able to support the growth of bacterial pathogen affecting man. There is one undisputed fact: without microbial involvement in the pulp and associated periapical tissues there would be no need for endodontic therapy.¹

Knowledge of the microorganisms associated with endodontic disease is necessary to develop a basic understanding of the disease process and a sound rationale for effective management of patient with endodontic infection.² Many microorganisms which are isolated from infected root canals of primary teeth are aerobic gram positive bacteria like Streptococcus sanguis, Streptococcus mutans, Streptococcus mitis and gram negative bacteria like Escherichia coli, Pseudomonas aeruginosa, Klebsiella.³ In the reported cases out of all the bacteria isolated, it is observed that facultative bacteria like Enterococcus faecalis is the most commonly isolated bacteria from the root canals failure cases.²

Intracanal medicaments are the materials which eliminate the bacteria thoroughly from the root canals thereby reducing the infection. Due to complex anatomy of the root canals mechanical instrumentation doesn't result in complete disinfection. Intracanal medicaments play an essential role in successful debridement and disinfection of the pulp space. Reduction of bacteria can be brought after instrumentation followed by use of intracanal medicaments which provide environment for conducive tissue repair.⁴

The purpose of this study is to evaluate the antibacterial efficacy of calcium hydroxide, 2% Chlorhexidine gluconate, triple antibiotic paste and aloe vera against anaerobic microorganisms in infected root canals of the primary teeth.

Materials and Methods:

The ensuing study was undertaken to evaluate the antibacterial efficacy of the various intracanal medicaments including Calcium Hydroxide, 2% Chlorhexidine gluconate, 3 Mix paste and Aloe vera gel over anaerobic microorganisms present in the infected root canals of the primary teeth. The study was conducted in the Post Graduate Department of Pedodontics and Preventive Dentistry, Seema Dental College and Hospital, Rishikesh.

Patients visiting the outpatient Department of Pedodontics and Preventive Dentistry, Seema Dental College and Hospital, Rishikesh with chief complaint of grossly decayed primary teeth were selected according to the following criteria.

Inclusion criteria:

1. Patients in the age group of 6-12 years with infected root canals of primary molars
2. Primary molars with abscess or draining sinus

Exclusion criteria:

1. Patients less than 6 years of age and more than 12 years of age.
2. Asymptomatic primary teeth
3. Permanent molar teeth

A total number of 120 microbial specimens were collected from primary molar teeth with atleast one necrotic canal, abscess or sinus tract. Sterile absorbent paper points of size compatible with root canal diameter were sequentially placed for 30 seconds. The retrieved paper points were immediately placed into the tube with thioglycolate. Pre-reduced thioglycolate tubes (0.5mg/l) were used as Transport medium. These samples were stored in the Glucose Broth Solution and incubated for 24 hours and then further it was placed into the anaerobic gas chamber for the identification or isolation of the anaerobic microorganisms with the help of the indicator tablets (Figure 5). After the anticipation of the bacterial colonies, the isolated bacterial strains were incubated at 37°C on blood agar plate (Figure 3); the discs were prepared for every individual group that is the experimental group and were placed on to the blood agar plate and then incubated for 24 hours at 37°C (Figure 4). After the incubation period the diameters of inhibitory halos were measured for the confirmation of the results (Figure 6,7).



SAMPLE COLLECTION USING STERILE PAPER POINTS (FIGURE 1)



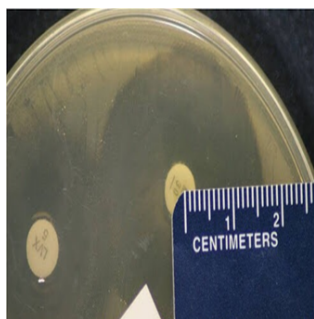
STORAGE OF SAMPLE IN THIOGLYCOLATE MEDIUM (FIGURE 2)



ISOLATION AND IDENTIFICATION OF MICROORGANISMS (FIGURE 3)



IDENTIFICATION OF THE INHIBITION ZONES (FIGURE 4)



MEASUREMENT OF THE INHIBITION ZONES (FIGURE 5)

RESULTS:

The readings obtained from the zones of inhibition against anaerobic microorganisms were subjected to statistical analysis. Mean and standard deviations were calculated from the measurements of inhibition halos for each identified predominant microorganisms.

Table 1: Mean Zone Of Inhibition Against Streptococcus

	Mean	Standard Deviation	f-value	p-value
3 Mix paste	30.90	4.802	1.266	0.000*
Aloevera	11.70	2.136		
2% Chlorhexidine	10.80	3.210		
Calcium Hydroxide	9.10	2.820		

The mean zone of inhibition produced against Streptococcus was highest by 3 Mix paste as compared to Aloevera, 2% Chlorhexidine and Calcium hydroxide using one-way ANOVA test. There was a statistical difference (p -value<0.05) in the mean zone of inhibition produced by the different groups against Streptococcus. (Graph 1)

Table 1.1: Bonferroni Pairwise Comparisons Test Of Streptococcus

	Streptococcus	Mean Difference	p-value
3 Mix paste	Aloevera	19.20	0.000*
	2% Chlorhexidine	20.10	0.000*
	Calcium Hydroxide	21.80	0.000*
Aloevera	2% Chlorhexidine	0.90	0.037*
	Calcium Hydroxide	2.60	0.000*
2% Chlorhexidine	Calcium Hydroxide	1.70	0.002*

The inter-group comparison of mean zone of inhibition against Streptococcus among 3 Mix paste, Aloevera, 2% Chlorhexidine gluconate and Calcium hydroxide was done using the Bonferroni Pairwise Comparisons test. The mean zone of inhibition was found to be significantly (p -value<0.05) higher by 3 Mix paste in comparison to Aloevera, 2% Chlorhexidine and Calcium hydroxide. Aloevera and 2% Chlorhexidine produced significantly (p -value<0.05) higher zone of inhibition in comparison to Calcium hydroxide whereas Aloevera had a significantly (p -value<0.05) higher zone of inhibition in comparison to 2% Chlorhexidine. (Graph 2)

Table 1.2: Comparison Of Streptococcus With Other Groups In Low, Medium And High Range

	Groups				Total	Pearson Chi-Square value	p-value
Streptococcus	2% Chlorhexidine	3 Mix paste	Aloevera	Calcium hydroxide			
Low (0.1-13.4)	24	0	21	24	69	107.38	0.000*
	80.0%	.0%	70.0%	80.0%	57.5 %		
Medium (13.5-26.8)	6	3	9	6	24		
	20.0%	10.0%	30.0%	20.0%	20.0 %		
High (26.9-45.0)	0	27	0	0	27		
	.0%	90.0%	0%	0%	22.5 %		
Total	30	30	30	30	120		
100.0%	100.0%	100.0%	100.0%	100.0%			

The zone of inhibition produced against Streptococcus by 3 Mix paste, Aloevera, 2% Chlorhexidine and Calcium hydroxide was compared using Chi-square test. There was a statistical difference (p -value<0.05) in the zone of inhibition produced by the different groups against Streptococcus. The high zone of inhibition was produced significantly (p -value<0.05) more by 3 Mix paste whereas low (0.1-13.4) was produced significantly (p -value<0.05) more by 2% Chlorhexidine, Aloevera and Calcium hydroxide

Table 2: Mean Zone Of Inhibition Against Lactobacilli

	Mean	Std. Deviation	f-value	p-value
3 Mix paste	24.60	5.934	2225.861	0.000*
Aloevera	33.20	5.261		
2% Chlorhexidine	11.70	2.136		
Calcium Hydroxide	10.80	3.210		

The mean zone of inhibition produced against Lactobacilli was highest in the case of Aloevera as compared to 3 mix paste 2% Chlorhexidine and Calcium hydroxide using one-way ANOVA test. There was a statistical difference (p -value<0.05) in the mean zone of inhibition produced by the different groups against Lactobacilli. (Graph 3)

Table 2.1: Bonferroni Pairwise Comparisons Test Of Lactobacilli

	Lactobacilli	Mean Difference	p-value
3 Mix paste	Aloevera	-8.60	0.000*

	2% Chlorhexidine	12.90	0.000*
	Calcium hydroxide	13.80	0.000*
Aloe vera	2% Chlorhexidine	21.50	0.000*
	Calcium hydroxide	22.40	0.000*
2% Chlorhexidine	Calcium hydroxide	0.90	0.037*

The inter-group comparison among 3 Mix paste, Aloe vera, 2% Chlorhexidine and Calcium hydroxide was done using the Bonferroni Pairwise Comparisons test. The mean zone of inhibition was found to be significantly (p-value<0.05) higher by Aloe vera in comparison to 3 mix paste, 2% Chlorhexidine and Calcium hydroxide. The values came out to be significant in relation to Lactobacilli. (Graph 4)

Table 2.2: Comparison Of Lactobacillus With Other Groups In Low, Medium And High Range

	Groups				Total	Pearson Chi-Square value	p-value
Lactobacillus	2% Chlorhexidine	3 Mix paste	Aloe vera	Calcium hydroxide			
Low (0.1-13.4)	21 70.0%	0 .0%	0 .0%	24 80.0%	45 37.5%	118.554	0.000*
Medium (13.5-26.8)	9 30.0%	21 70.0%	3 10.0%	6 20.0%	39 32.5%		
High (26.9-45.0)	0 .0%	9 30.0%	27 90.0%	0 .0%	36 30.0%		
Total	30 100.0%	30 100.0%	30 100.0%	30 100.0%	120 100.0%		

The zone of inhibition produced against Lactobacillus by 3 Mix paste, Aloe vera, 2% Chlorhexidine and Calcium hydroxide was compared using Chi-square test. There was a statistical difference (p-value<0.05) in the zone of inhibition produced by the different groups against Lactobacillus. The high (26.9-45.0) zone of inhibition was produced significantly (p-value<0.05) more by Aloe vera, medium (13.5-26.8) was produced by 3 Mix paste whereas low (0.1-13.4) was produced significantly (p-value<0.05) more by 2% Chlorhexidine and Calcium hydroxide.

Table 3: Mean Zone Of Inhibition Against Clostridium Species

	Mean	Std. Deviation	f-value	p-value
3 Mix paste	30.90	4.802	1149.038	0.000*
Aloe vera	11.70	2.136		
2% Chlorhexidine	10.80	3.210		
Calcium hydroxide	10.30	2.984		

The mean zone of inhibition produced against clostridium spp was highest in the case of 3 Mix pastes as compared to Aloe vera, 2% Chlorhexidine and Calcium hydroxide using one-way ANOVA test. There was a statistical difference (p-value<0.05) in the mean zone of inhibition produced by the different groups against Clostridium species. (Graph 5)

Table 3.1: Bonferroni Pairwise Comparisons Test Of Clostridium Species

	Clostridium Species	Mean Difference	p-value
3 Mix paste	Aloe vera	19.20	0.000*
	2% Chlorhexidine	20.10	0.000*
	Calcium hydroxide	20.60	0.000*
Aloe vera	2% Chlorhexidine	0.90	0.037*
	Calcium hydroxide	1.40	0.01
2% Chlorhexidine	Calcium hydroxide	0.50	0.01

The inter-group comparison among 3 Mix paste, Aloe vera, 2% Chlorhexidine and Calcium hydroxide was done using the Bonferroni Pairwise Comparisons test which came out to be significant in relation to 3 mix pastes and insignificant in relation to 2% Chlorhexidine. (Graph 6)

Table 3.2: Comparison Of Clostridium With Other Groups In Low, Medium And High Range

	Groups				Total	Pearson Chi-Square value	p-value
Clostridium	2% chlorhexidine	3 Mix paste	Aloe vera	Calcium hydroxide			

Low (0.1-13.4)	24 80.0%	0 .0%	21 70.0%	27 90.0%	72 60.0%	110.714	0.000*
Medium (13.5-26.8)	6 20.0%	3 10.0%	9 30.0%	3 10.0%	21 17.5%		
High (26.9-45.0)	0 .0%	27 90.0%	0 .0%	0 .0%	27 22.5%		
Total	30 100.0%	30 100.0%	30 100.0%	30 100.0%	120 100.0%		

The zone of inhibition produced against Clostridium by 3 Mix paste, Aloe vera, 2% Chlorhexidine and Calcium hydroxide was compared using Chi-square test. There was a statistical difference (p-value<0.05) in the zone of inhibition produced by the different groups against Clostridium. The High (26.9-45.0) zone of inhibition was produced significantly (p-value<0.05) more by 3 Mix paste whereas low (0.1-13.4) was produced significantly (p-value<0.05) more by 2% CHX, Aloe vera and Ca(OH)².

Table 4: Mean Zone Of Inhibition against E.Faecalis

	Mean	Std. Deviation	f-value	p-value
3 Mix paste	11.70	2.136	978.598	0.000*
Aloe vera	10.80	3.210		
2% Chlorhexidine	33.20	5.261		
Calcium hydroxide	10.80	3.210		

The mean zone of inhibition produced against E.faecalis was highest in the case of 2% Chlorhexidine as compared to 3 mix paste, Aloe vera and Calcium hydroxide using one-way ANOVA test. There was a statistical difference (p-value<0.05) in the mean zone of inhibition produced by the different groups against E. faecalis. (Group 7)

Table 4.1: Bonferroni Pairwise Comparisons Test Of E.Faecalis

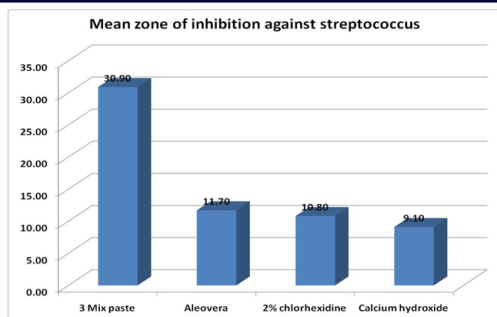
E. faecalis	E. faecalis	Mean Difference	p-value
3 Mix paste	Aloe vera	0.90	0.037*
	2% Chlorhexidine	-21.50	0.000*
	Calcium hydroxide	0.90	0.037*
Aloe vera	2% Chlorhexidine	-22.40	0.000*
	Calcium hydroxide	0.00	0.000*
2% Chlorhexidine	Calcium hydroxide	22.40	0.000*

The inter-group comparison among 3 Mix paste, Aloe vera, 2% Chlorhexidine and Calcium hydroxide was done using the Bonferroni Pairwise Comparisons test which came out to be insignificant in relation to Aloe vera when tested with Calcium hydroxide. (Graph 8)

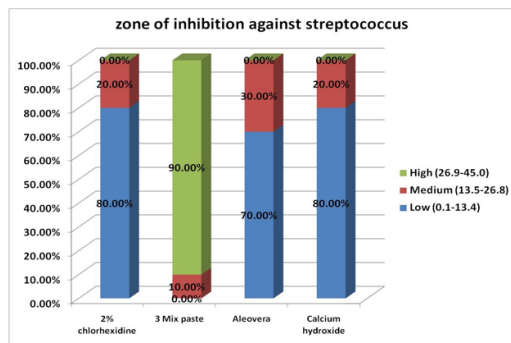
Table 4.2: Comparison Of E.Faecalis with Other Groups In Low, Medium And High Range

	Groups				Total	Pearson Chi-Square value	p-value
E. faecalis	2% chlorhexidine	3 Mix paste	Aloe vera	Calcium hydroxide			
Low (0.1-13.4)	0 .0%	21 70.0%	24 80.0%	24 80.0%	69 57.5%	107.34	0.000*
Medium (13.5-26.8)	3 10.0%	9 30.0%	6 20.0%	6 20.0%	24 20.0%		
High (26.9-45.0)	27 90.0%	0 .0%	0 .0%	0 .0%	27 22.5%		
Total	30 100.0%	30 100.0%	30 100.0%	30 100.0%	120 100.0%		

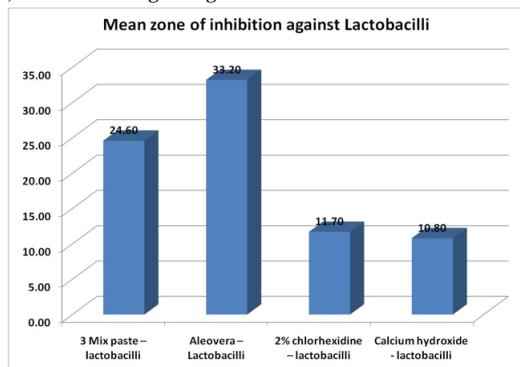
The zone of inhibition produced against E. faecalis by 3 Mix paste, Aloe vera, 2% chlorhexidine and Calcium hydroxide was compared using Chi-square test. There was a statistical difference (p-value<0.05) in the zone of inhibition produced by the different groups against E. faecalis. The high (26.9-45.0) zone of inhibition was produced significantly (p-value<0.05) more by 2% CHX whereas low (0.1-13.4) was produced significantly (p-value<0.05) more by 3 Mix Paste, Aloe vera and Ca(OH)².



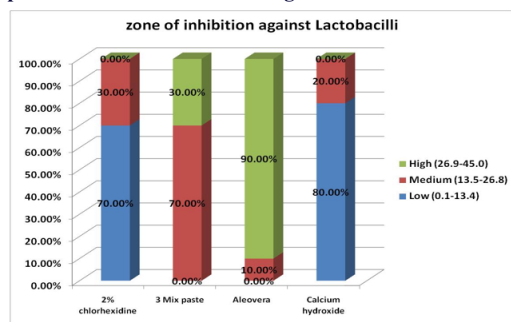
Graph 1: Mean Zone Of Inhibition Against Streptococcus



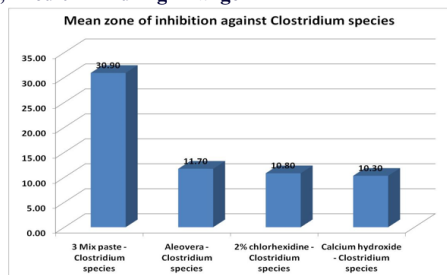
Graph 2: comparison of zone of inhibition against streptococcus in low, medium and high range



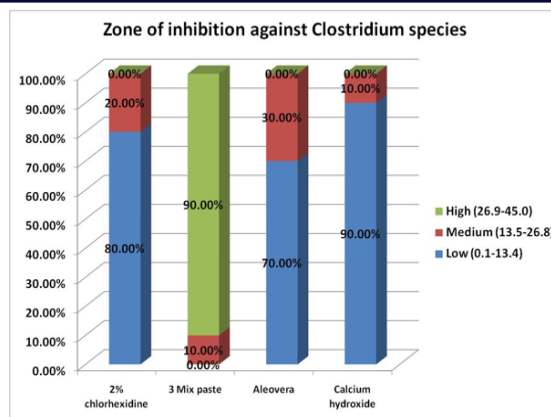
Graph 3: Mean Zone of Inhibition against Lactobacilli



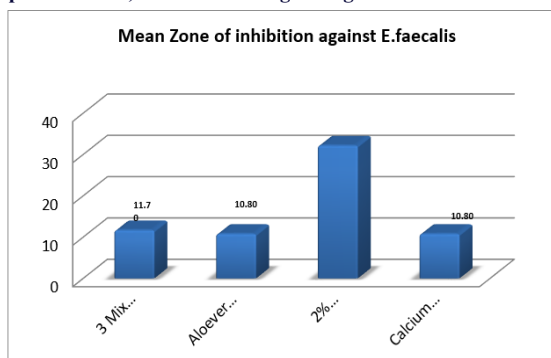
Graph 4: Comparison Of Zone Of Inhibition Against Lactobacilli In Low, Medium And High Range



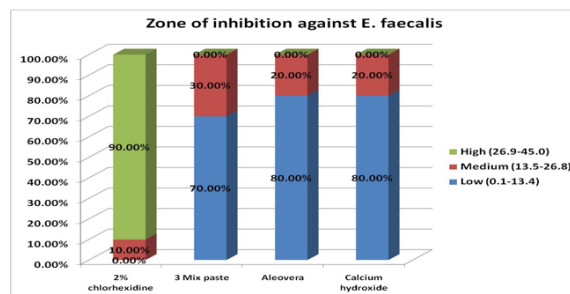
Graph 5: Mean Zone Of Inhibition Against Clostridium Species



Graph 6: Comparison Of Zone Of Inhibition Against Clostridium Species In Low, Medium And High Range



Graph 7: Mean Zone Of Inhibition Against E.Faecalis



Graph 8: Comparison Of Zone Of Inhibition Against E.Faecalis In Low, Medium And High Range

Discussion:

One of the major purposes of pediatric dentistry is to maintain primary teeth in anatomical and functional conditions up to their physiological exfoliation and eruption of permanent teeth. It is fundamental to prevent changes in chewing, speech and phonetics, as well as for maintenance of the dental arch length and esthetics, and prevention of deleterious oral habits. In the absence of endodontic treatment and bacterial control, inflammatory mediators are released and can compromise the vitality of dental pulp leading the tissue to dystrophic calcification or to necrosis.⁵ Endodontic infections are symptomatic or asymptomatic inflammatory diseases caused by microbial infection of the root canal system (Siqueira, 2002).⁶ Oral microbial flora may give different isolation & identification of the microbes in infected primary root canals such as Streptococci, Peptostreptococcus, Lactobacilli, Propionibacterium, Actinomyces, Eubacterium, Veillonellaparuvala, Bacteroides, Fusobacterium etc.⁷

In the present study, the anaerobic microorganisms were isolated from the samples of infected root canals of the primary teeth including Peptostreptococcus, Prevotella, Actinomyces, Eubacterium, Fusobacterium with a predominance of Enterococcus Faecalis, Streptococcus Intermedius, Lactobacillus and Clostridium species which is in accordance with the study of Pazelli et al (2003)⁷ who found that in human primary root canals with necrotic pulp and periapical lesions, the infection was polymicrobial with a predominance of

streptococci, anaerobic micro-organisms and black pigmented bacilli. Yesilsoy et al (1995)⁸ concluded that to eliminate bacteria from the root canals predictably, the supporting action of a disinfecting agent is mandatory. In the present study, predominated microorganisms were subjected to various intracanal medicaments which were Calcium Hydroxide, 2% Chlorhexidine gluconate, 3 Mix paste and Aloe vera and their zones of inhibition (Halos) were calculated to assess the level of efficacy of various medicaments towards the anaerobic microorganisms onto the blood agar using the disc diffusion method.

According to the present study, 2% CHX showed highest antimicrobial activity against *E. faecalis* which is in accordance with the study conducted by E. Cruz (2004)⁹. Schafer and Bossmann (2009)⁹ also found the same results with 2% chlorhexidine (CHX) that it has an excellent efficacy against *E. faecalis*. This finding coincides with an in vitro study by Gomes et al (2003)¹⁰ who reported that chlorhexidine completely inhibited the growth of *E. faecalis*. These observations confirm recent reports that 2% chlorhexidine has a marked effect against *E. faecalis* which is in accordance with the present study. Lowest antimicrobial activity against *E. faecalis* was shown by Calcium Hydroxide and Aloe vera.

Bhardwaj *et al*¹¹ found in their study, 2% CHX gel completely inhibited the growth of *E. faecalis*, while Aloe vera and calcium hydroxide inhibited bacterial growth by 78.9% and 64.3% respectively which favours the results of the present study.

In the current study, antibacterial efficacy of 3 Mix paste showed highest and most significant results and effectiveness against *Streptococcus intermedius* in accordance with the study by Sato et al (1996)¹² who found that this drug combination was very effective in killing anaerobic bacteria in the deep layers of root canal dentin. It was found in the present study that 3-mix paste showed highest antibacterial activity against *Clostridium ramosum* which is in contrast with the study conducted by Velasco-Loera N et al (2012) who found 3-mix paste to exhibit largest inhibition zones against *P. acnes*, *G. morbillorum* but no bactericidal activity against *C. albicans* and *C. ramosum* in primary root canals.¹³

In endodontics because of the cytotoxic reactions of most of the commercial intracanal medicaments used and their inability to eliminate bacteria from dentinal tubules, there is a recent increase in trend to use biologic medication extracted from natural plants. Among the four intracanal medicaments used in the study, Aloe vera (herbal product) was used in the study. Aloe vera (synonym: *Aloe barbadensis* Miller) belongs to the Liliaceae family.¹⁴ Aloe vera is a kind of plant that its leaf is composed of two major parts. The outer green rind contains vascular bundles and the inner colorless part, named as aloe gel, includes the parenchymal cells which contain viscos clear liquid. The aloe gel consists of 99.5% water. The remaining solid material including mineral, vitamins, enzymes, polysaccharides, phenolic compounds and organic acids.¹⁵

Aloe vera is found to possess good wound healing activity. Aloe vera is a potent anti inflammatory agent. It exhibits potent anti viral and anti tumor activity. Aloe vera possesses good anti bacterial and anti fungal activity. The possible reason for antimicrobial action of aloe vera could be the presence of 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids. In dentistry, Aloe vera is used in cases of Aphthous ulcers, Lichen planus, and Alveolar osteitis. Its use has been documented in root canal treatment as a sedative dressing, healing promoter and file lubricant.¹³

Fani M¹¹ found that aloe vera gel shows bactericidal activity against both cariogenic and periodontopathic bacteria. The Aloe Vera gel was initially evaluated by the disc diffusion method using 20 isolates of *S. mutans* in which undiluted Aloe vera gel produced significant growth inhibition zones against all the oral bacteria tested.¹¹ In the present study, it was found to be most effective against *Lactobacillus* species which was in contradiction to the study by Fani M.

Lactobacillus species are usually resistant against most of the intracanal medicaments but Aloe vera shows highest zone of inhibition against it, hence proving the beneficial effects of Aloe vera against resistant microorganisms. 3 Mix paste also seems to have moderate inhibitory effect against *Lactobacillus* species. Ersin and colleagues (2006)¹⁶ could not demonstrate a significant superior effect of Chlorhexidine on *Lactobacillus* which is in accordance with the

present study as 2% CHX shows minimal effect against *Lactobacillus* in the study. Always in the diseased tissues, biochemical signaling takes place which involves three biological phases: inflammation, connective tissue degradation, and alveolar bone turnover contributing to the clinical morbidity.¹⁷ Aloe vera showed lowest zone of inhibition against *E. faecalis* in the present study which contrast the study of Chandra S et al who investigated Aloe vera gel on different strains of bacteria and yeasts and concluded that chloroform extract of Aloe vera showed significant zone of inhibition against *E. faecalis*. Hence, Aloe vera has shown excellent antimicrobial effect against resistant microorganisms found in pulp space by demonstrating marked zones of inhibition against *Lactobacillus* and *Streptococcus intermedius*.

Till date no study has been conducted to comparatively evaluate the antimicrobial activity of Calcium hydroxide, 2% Chlorhexidine, 3 Mix paste and Aloe vera, against anaerobic microorganisms in the infected root canals of primary teeth. Hence, this study was undertaken to effectively compare the antimicrobial activity of the above mentioned intracanal medicaments against anaerobic microorganisms present in the infected root canals of primary teeth.

REFERENCES:

- 1) Siqueira F, Rocas I. Exploiting molecular methods to explore endodontic infections; Part I- Current molecular technologies for microbiological diagnosis. J Endod 2005; 31:6.
- 2) Menezes MM, Valera MC, Jorge AO et al. In vitro evaluation of the effectiveness of irrigants and intracanal medicaments on microorganisms within root canals. J Endod 2004; 37(5):311-19.
- 3) Peculene V, Maneliene R, Balciakonyte E et al. Microorganisms in root canal infections: A Review. Stomatol Baltic Dent and Maxillofacial J 2008; 10:4-9.
- 4) Siqueira F J and Rocas I R. Diversity of endodontic Microbiota Revisited. J Dent Res 2009; 88(11):969-81.
- 5) Pimenta HC, Borges AH, Bandeca MC. Antimicrobial activity of Filling Materials Used in Primary Teeth Pulpotomy. J Int Oral Health 2015; 7(4): 54-57.
- 6) Peculene V, Maneliene R, Balciakonyte E, Drukteinis S, Rutkunas V. Microorganisms in root canal infections: a review. Stomatol, Baltic Dent and Maxillofacial J 2008; 10:4-9.
- 7) Rana V, Baba SM, Pandey A. Bacteriology of infected deciduous root canal- a review. People's Journal of Scientific Research 2009; 2(2):45-48.
- 8) Ercan E, Ozekinci T, Atakul F et al. Antibacterial activity of 2% Chlorhexidine Gluconate and 5.25% Sodium Hypochlorite in Infected Root Canal: In Vivo Study. J Endod 2004; 30(2):18-22.
- 9) Lee Y, Han SH, Hong SH. Antimicrobial Efficacy of a Polymeric Chlorhexidine Release Device Using In Vitro Model of Enterococcus faecalis Dental Tubule Infection. J Endod 2008; 34: 7-12.
- 10) Bhardwaj A, Ballal S, Velmurugan N. Comparative evaluation of the antimicrobial activity of natural extracts of Morinda citrifolia, papain and aloe vera (all in gel formulations), 2% Chlorhexidine gel and calcium hydroxide against Enterococcus faecalis: An in vitro study. J Cons Dent 2012; 15(3):293-97.
- 11) Awawdeh L, Al-Beitawi M, Hammad M. Effectiveness of propolis and calcium hydroxide as a short-term intracanal medicament against Enterococcus faecalis: A laboratory study. Aust Endod J 2009; 35: 52-58.
- 12) Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 2000 1994; 5:78-111.
- 13) Evanov C, Liewehr F, Thomas B, Buxton et al. Antibacterial Efficacy of Calcium Hydroxide and Chlorhexidine Gluconate Irrigants at 37°C and 46°C. J Endod 2004; 30: 9-11.
- 14) Er K, Kustarci A, Ozan U, Tasdemir T. Nonsurgical endodontic treatment of dens invaginatus in a mandibular premolar with large periradicular lesion. J Endod 2007; 33:e322-24.
- 15) Monica B, Monisha R. Aloe vera in dentistry- a review. IOSR J Dent and Med Sci 2014; 13(12): 18-22.
- 16) Gomes BFPA, Pinheiro ET, Gade-Neto CR, Sousa ELR, Ferraz CCR, Zaia AA. Microbiological examination of infected dental root canals. Oral Microbiol Immunol 2004; 19:71-76.
- 17) Kaul M, Shukla S, D.K Suresh. A cursory glance on biomarkers for bone in health and disease. International Journal of Dental Research & Development 2016; 6(4):49-58. (https://www.researchgate.net/publication/328333409_A_CURSORY_GLANCE_ON_BIOMARKERS_FOR_BONE_IN_HEALTH_AND_DISEASE).