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Comparative evaluation of antibacterial efficacy of chlorhexidine gluconate, fenugreek (*Trigonella foenum*) and Fennel (*Foeniculum vulgare*) as intracanal irrigant on isolated bacteria from infected primary tooth

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Abstract
Aim: To evaluate antibacterial efficacy of Chlorhexidine gluconate (2%), Fenugreek (*Trigonella foenum*) and Fennel (*Foeniculum vulgare*) as intracanal irrigant on isolated bacteria from infected primary tooth.
Materials and methods: Thirty patients were selected based on inclusion and exclusion criteria. After rubber dam isolation access opening was done and collection of sample using absorbent paper point was done. Samples were processed for microbiological procedure and isolation of different species of bacteria was done. All the individual species were subjected to antibacterial sensitivity for three irrigants.
Results: Different species of obligatory and facultative anaerobes were isolated mainly *Peptostreptococcus* colonies of obligatory anaerobic gram positive cocci followed by facultative anaerobe *E. faecalis*, followed by gram negative *Bacilli* such as *P. intermedia*, *Porphyromonas* species, *Bacteroides* species, and *Fusobacterium* species. Facultative Gram-positive anaerobic cocci such as *Streptococcus pyogenes*, *S. sobrinus*, and *Staphylococcus aureus* were also found but were comparatively less in number. These were subjected to antibacterial sensitivity against three irrigants. The results statistically analysed using Pearson's Chi-square test for two non-parametric data and proportional comparisons were done using Z test for two sample proportion. Chlorhexidine was found most sensitive, followed by Fennel extract and least sensitive is Fenugreek Extract for facultative as well as obligatory anaerobes.
Conclusion: The bacterial profile in infected primary teeth consists of mainly obligatory anaerobes *Peptostreptococcus* colonies, followed by *E. Faecalis* and black pigmented colonies. Amongst two herbal irrigant, fennel can act a potent herbal substitute for chlorhexidine as intracanal irrigant in infected primary teeth.

Keywords: Infected primary teeth, anaerobic bacteria, intracanal irrigant, chlorhexidine gluconate, fennel, fenugreek, antibacterial sensitivity

Introduction
The success, reliability and longevity of endodontic treatment in primary teeth depends on complete elimination of bacteria from root canal before obturation [1]. It has been clearly demonstrated in animal models and human studies that microorganisms play a key role in the development and perpetuation of pulp and periapical diseases [2].
Endodontic treatment of primary teeth with necrotic pulp is routine in dental practice. It is fundamental that the dentist be aware of the microbiota in primary teeth so that adequate antimicrobial agents may be used to eliminate these pathogens [3].
For complete elimination of infection from the roots of primary teeth, irrigation is an important step for the success of pulpectomy [4]. Most of the commercial intra-canal medicaments used in endodontics such as sodium hypochlorite and chlorhexidine cause cytotoxic reactions, have side effects, unpleasant taste, foul smell, allergic potential, and because of their inability to eliminate bacteria from dentinal tubules, recent medicine has turned its attention to the usage of biologic medication prepared from natural plants [5].

Although the literature for permanent teeth is numerous, but there is a need for the study of herbal irrigants in pediatric dentistry as an alternative for commercial intra-canal irrigants to avoid unwanted side effects.

Hence the present study was done to find the bacterial profile in primary teeth and to study the antibacterial effects of two new herbal products, fennel (2%) and fenugreek (2%), which has not been tested as intra-canal irrigant in primary and permanent teeth till date and compare them with chlorhexidine (2%).

Materials and method

The study was conducted in Department of Pediatric and Preventive Dentistry at Sri Aurbindo College of Dentistry Indore. Thirty patients undergoing pulpectomy were selected based on the selected inclusion and exclusion criteria.

Inclusion criteria

- Patient aged 3-10 years.
- Primary teeth with necrotic pulp, chronic abscess, and/or sinus tract.

Exclusion criteria-

- Teeth with more than two-third loss of root structure evidence of root resorption and/or mobility.
- Teeth with antibiotic usage in past 4 weeks.
- Patient with systemic diseases.

Extract preparation: Pure extracts of fennel and fenugreek were obtained from local shop (Gangaram mohanlal ayurvedic shop, Indore). Powdered spices were soaked in 200 ml of 95% ethanol and kept at room temperature, macerated for 1 days, and were filtered. The filtrate was heated at 40-50 °C using water baths till the ethanol evaporated and until thick paste was formed. The thick paste was considered as 100% concentration of extract. These extracts (Fig 1) were stored at 4 °C in refrigerator. For testing the desired concentration of 2% was obtained by dissolving 2 gm of pure hydroalcoholic extracts in 100 ml of ethanol.

Fig 1: Extract of fennel and fenugreek

Procedure

After administering local anesthesia using 2% lignocaine with 1:80,000 adrenaline, rubber dam isolation was done. A standard access cavity preparation was made followed by working length determination. One absorbent point was taken till the apex per canal and was held there for 30seconds. After removing from the canals paper points were directly transferred into a tube containing thioglycolate broth. This tube was processed within 30 min of sample collection according to the standard microbiological protocol and was kept in incubator where growth was checked after 48 hours.

After 48 hours the turbidity of bacteria obtained in the test tubes was inoculated on blood agar plates using inoculating loops. The plates were instantly transferred to anaerobic jar were anaerobic environment was created using anaerobic gas- pack (Hi-media), which was again incubated for 48 hrs. Bacterial growth was obtained on the plates after 48 hours (Fig 2).

From each plate bacterial strains were identified based on Gram staining and was classified by colony morphology. Following which all isolated microbial strains were subjected to antibiotic sensitivity testing using kanamycin, colistin, metronidazole, vancomycin and ampicillin on agar plates for identifying specific species of bacteria.

After identification of bacterial species, each species were tested against three irrigants using disk diffusion method in which sterile filter disks were used for dispensing the irrigants.

The agar media plates were inoculated with the organisms by even streaking of the swab over the entire surface of the plate 3 times, rotating the plate approximately 60° after each application to ensure an even distribution of the inoculums. 10 ml volume of each of the hydroalcoholic extract prepared in saturated concentrations was dispensed into the sterile disk of inoculated plates. The sensitivity or resistance of the isolated bacteria was determined after 48 hours incubation by the zone of inhibition (Fig 3) around each disk.

Fig 2: Bacterial growth after 48 hrs.

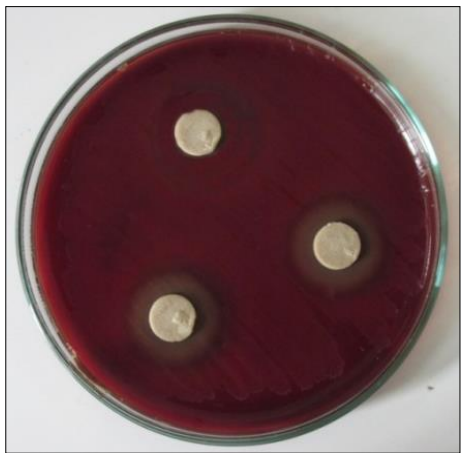

Fig 3: Zone of inhibition around sterile disk containing respective test irrigant

Results

The different strains of bacteria were isolated from the samples which were divided into different groups based on whether Gram-positive or Gram-negative, facultative or obligatory anaerobes, cocci or Bacilli.

The Table 1 shows isolated bacteria and respective number of samples which were obtained in present study. Sensitivity of three irrigants was recorded in each sample. The data was analyzed using IBM SPSS Ver. 20.0.0 software. The association between two non-parametric data was done using Pearson's Chi-square test and proportional comparisons were done using Z test for two sample proportion. A P value of < 0.05 was taken as statistically significant. Results were tabulated for obligatory and facultative anaerobes.

Association of sensitivity and resistance between the three irrigants groups for both the group of bacteria was obtained and tabulated in table 2.

Majority of the samples of Chlorhexidine group and Fennel Extract group were sensitive, while in the Fenugreek Extract group, majority of the samples were resistant for obligatory anaerobes. Similar results were obtained for facultative anaerobes in Chlorhexidine group and Fennel extract group. Facultative anaerobes showed more sentivity to fenugreek extract as compared to obligatory anaerob

Table 1: Classification of isolated species

Type of bacterial strain	Number of samples from which they were obtained out of 30
Obligatory Anaerobes	
Gram Negative Bacilli	
Bacteroid	15
Black pigmented colonies	11
Gram Positive Cocci	
Peptostreptococcus	25
Facultative Anaerobes	
Gram Positive Cocci	
E. Faecalis	18
S. Pyogens	16
S. Sobrinus	10
S. Aureus	5

Table 2: Association of sensitivity and resistance between the three groups for Obligatory Anaerobes and facultative anaerobes

Irrigant	Obligatory Anaerobes			Facultative Anaerobes		
	Chlorhexidine (N=42)	Fennel Extract (N=42)	Fenugreek Extract (N=42)	Chlorhexidine (N=42)	Fennel Extract (N=42)	Fenugreek Extract (N=42)
Sensitivity	36(85.71%)	34(80.95%)	4(9.52%)	43(84.31%)	40(78.43%)	22(43.13%)
Resistance	6(14.29%)	8(19.05%)	38(90.48%)	8(15.69%)	11(21.57%)	29(56.87%)

$\chi 2$ value = 63.131, df=2, P value = 0.000*

The association of sensitivity and resistance in relation to the groups was obtained. The chi-square value obtained was 63.131, with a degree of freedom of 2. The P value obtained is < 0.05, which is statistically significant, showing that there is an association between the sensitivity, resistance and the groups for obligatory anaerobes.

The Table 3, shows the proportional comparison of sensitivity among the three groups for obligatory anaerobes.

There is no statistically significant difference in the proportions of sensitive samples between Chlorhexidine and Fennel Extract group (P>0.05), while statistically significant difference in proportions was seen between Fennel Extract-Fenugreek The table 4 shows the proportional comparison of sensitivity among the three groups.

Chlorhexidine is most sensitive, followed by Fennel extract and least sensitive is Fenugreek Extract for facultative as well as obligatory anaerobes.

Table 3: The proportional comparison of sensitivity among the three groups for obligatory anaerobes

Group	Proportion	'Z' Value	P Value
Chlorhexidine	36 / 42	0.59	0.557, NS
Fennel Extract	34 / 42		
Fennel Extract	34 / 42	10.81	0.000*
Fenugreek Extract	4 / 42		
Chlorhexidine	36 / 42	9.44	0.000*
Fenugreek Extract	4 / 42		

*-Significant

Table 4: The proportional comparison of sensitivity among the three groups for facultative anaerobes

Group	Proportion	'Z' Value	P Value
Chlorhexidine	43 / 51	0.77	0.444, NS
Fennel Extract	40 / 51		
Fennel Extract	40 / 51	3.92	0.000*
Fenugreek Extract	22 / 51		
Chlorhexidine	43 / 51	4.79	0.000*
Fenugreek Extract	22 / 51		

*****Significant

Discussion

Dental caries is the most common reason for the early loss of deciduous tooth. In this process, the oral microflora plays a very crucial role. The colonization of the bacteria along with the other contributing factors is responsible for the progression of caries leading to pulpal inflammation, infection, swelling, abscesses, etc. For this reason, the identification of the oral microbiota found in the primary teeth is important.

In the present study, it was observed that the most predominant organisms isolated from the infected, abscessed primary teeth were the *Peptostreptococcus* colonies of obligatory anaerobic gram positive cocci followed by facultative anaerobe *E. faecalis*, followed by *gram negative Bacilli* such as *P. intermedia*, *Porphyromonas* species, *Bacteroides* species, and *Fusobacterium* species. Facultative Gram-positive anaerobic

cocci such as *Streptococcus pyogenes*, *S. sobrinus*, and *Staphylococcus aureus* were also found but were comparatively less in number. Toyoshima *et al*. and Sato *et al* [5, 6] also reported that in root canals of primary teeth with necrotic pulp and periapical lesions which need retreatment had a polymicrobial infection with predominance of anaerobic microorganisms.

Tomic-Karovic and Jelinek (1971) found black pigmented microorganisms in 36% of the root canals of deciduous teeth with necrotic pulp, which is also in relevance to present study [7].

The endodontic instrumentation cannot effectively eliminate the microflora from the root canals of primary teeth mechanically owing to their anatomic complexity. The dependence on irrigating solutions for endodontic success becomes more crucial, especially in primary teeth due to thin dentinal walls, complex morphology, and irregularity of root canal system [8].

Chlorhexidine is basically a synthetic cationic bis-guanide that consists of two symmetric 4-cholorophenyl rings and two biguanide groups which are connected by a central hexamethylene chain. It is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through some type of active or passive transport mechanism. Its efficacy is because of the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls, thereby altering the cells osmotic equilibrium [9].

Although chlorhexidine does not have many disadvantages as sodium hypochlorite, there are studies showing cytotoxic effects of chlorhexidine [10] and even it does not taste good for use amonst children and also causes teeth discoloration.

The hydroalcoholic extract of Fennel showed equivalent antibacterial sensitivity as chlorhexidine. Except for some Gram-positive facultative anaerobes fennel showed a wide spectrum of antibacterial effect against the organisms most commonly seen in the infected primary teeth such as black pigmented microorganisms, *P. intermedia*, *Peptostreptococcus*, and *E. faecalis* that were isolated in the present study.

Fennel belongs to Apiaceae family with scientific name *Foeniculum vulgare* Mill. It is an herbaceous and aromatic plant, with a height of 1 to 2 meters. Fennel is a perennial herb. Fennel has antibacterial activity due to compounds such as, linoleic acid, undecanal, 1, 3-benzenediol, oleic acid and 2,4-undecadienal. Fennel has 5-hydroxy-furanocoumarin which has important role antibacterial activity of this plant [11].

Fenugreek did not show similar results as fennel and chlorhexidine but had antibacterial activity against some species of facultative anaerobes. Fenugreek seeds contain lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline [12]. According to the results of present study fennel can be used as efficient herbal irrigant.

Conclusion

The micro-organisms prevalent in primary teeth with necrosis, abscess, and/or sinus tract include *Peptostreptococcus*, *Porphyromonas* species, *P. intermedia*, *B. fragilis*, *Fusobacteri*um, as obligate anaerobes. *Streptococcus pyogenes*, *S. sobrinus*, *E. faecalis*, and *S. aureus*, *as Facultative anaerobes* in the present study fennel was found to show promising results as intracanal irrigant. However, the results of the present study can be justified by a larger sample size and use of some other antibacterial tests before its clinical application in dentistry.

References

1. El Karim I, Kennedy J, Hussey D. The antimicrobial effects of root canal irrigation and medication. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007; 103:560-9.
2. Mohammadi Z. An update on the antibiotic-based root canal irrigation solutions. Iran Endod J. 2008; 3:1-7.
3. Da Silva LA, Nelson-Filho P, Faria G, de Souza-Gugelmin MC. Ito IY. Bacterial profile in primary teeth with necrotic pulp and periapical lesions. Braz Dent J. 2006; 17:144-8.
4. Jolly M, Singh N, Rathore M, Tandon S, Banerjee M. Propolis, and commonly used intracanal irrigants: Comparative evaluation of antimicrobial potential. J Clin Pediatr Dent. 2013; 37:243-9.
5. Toyoshima Y, Fukushima H, Inoue JI, Sasaki Y, Yamamoto K, Katao H, Ozaki K, Moritani Y, Saito T, Hieda T *et al*. A bacteriological study of periapical pathosis on deciduous teeth. JPN Dent J. 1988; 26:449-458.
6. Sato T, Hoshino E, Uematsu H, Noda T. Predominant obligate anaerobes in necrotic pulps of human deciduous teeth. Microb Ecol Health Dis. 1993; 6:269-275.
7. Tomic-Karovic K, Jelinek, E. Comparative study of the bacterial flora in the surroundings, the root canals and sockets of deciduous molars Int Dent J. 1971; 21: 375 388.
8. Thomas AM, Chandra S, Chandra S, Pandey RK. Elimination of infection in pulectomized deciduous teeth A short-term study using iodoform paste J. Endod 1994; 20:233-5.
9. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. International endodontic journal. 2009; 42(4):288-302.
10. Tatnall FM, Leigh IM, Gibson JR. Comparative study of antiseptic toxicity on basal keratinocytes, transformed human keratinocytes and fibroblasts. Skin Pharmacology. 1990; 3:157–63.
11. Kooti W, Moradi M, Akbari SA, Sharafi-Ahvazi N, Asadi-Samani M, Ashtary-Larky D. Therapeutic and pharmacological potential of *Foeniculum vulgare* Mill: a review. J Herb Med Pharmacol. 2015; 4(1):1-9.
12. Michael D, Kumawat D. Legend and archeology of fenugreek, constitutions and modern applications of fenugreek seeds. Int Symp. 2003; 41-42.