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KEY WORDS: Calcium Hydroxide, chlorhexidine, Tulasi, Enterococcus faecalis, primary teeth, permanent teeth.

AN IN VITRO EVALUATION OF ANTIMICROBIAL EFFICACY OF CALCIUM HYDROXIDE IN COMBINATION WITH CHLORHEXIDINE AND TULASI AGAINST *ENTEROCOCCUS FAECALIS*

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ABSTRACT

Introduction: *Enterococcus faecalis*is a gram positive anaerobe very resistant to irrigation and biomechanical preparation. For this reason, intra canal medication between appointments is recommended to further reduce bacteria in the root canal system. Here in the present study the combination of Ca(OH)2 and chlorhexidine and herbal extract tulsiis used.

Aims & objectives: To evaluate whether CAOH & CHX combination is better than tulsi in primary and permanent teeth against e.faecalis.

Materials and methodology: Calcium hydroxide paste(CAL-EXCEL), 1% Chlorhexidine, Tulsi extract (95%), 60 teeth which are 30 primary and 30 permanent teeth ,materials used in the present study, Contamination of root canals *E. faecalis*(ATCC 29212) was used as the test organism,

The 60 samples were randomly separated into three experimental groups (n = 20) .One way Anova,Two way Anova, T test and Multiple comparision post hoc tests are used for the statistical analysis.

Conclusion: study calcium hydroxide and chlorhexidine combination is more efficient than tulsi.

INTRODUCTION:

A favourable outcome of the endodontic treatment of teeth with apical periodontitis depends on effective control of the root canal infection (1,2). Chemo-mechanical cleaning and shaping of the root canal can greatly reduce the number of microorganisms but not completely eliminate them (3).The need of medication increases in those cases where an infection resists regular treatments and the therapy cannot be successfully completed owing to presence of pain and exudation (4). Therefore, an effective antimicrobial treatment protocol should be used to reduce bacterial insult to minimum allowing host's defence system to take over and provide a favourable environment for healing (5,6).

*Enterococcus faecalis*is a gram positive anaerobe, and the prevalence of *E. Faecalis* in failed endodontic cases ranges between 24 and 70 percent.(4) Like other facultative anaerobes ,*E.faecalis*is very resistant to irrigation and biomechanical preparation. For this reason, intra-canal medication between appointments is recommended tofurther reduce bacteria in the root canalsystem.

Enterococcus faecalis is part of normal oral flora, and is found in small numbers in uninstrumented infected root canals. Its role in endodontic infections was and still is a big issue in root canal therapy. E. faecalis is the most prevalent species isolated from root canals of previously root-filled teeth with chronic apical periodontitis (7).

The excellent biologic and antimicrobial properties of calcium hydroxide have made this medication the choice for the intra-canal dressing of infected root canals. It is bactericidal and neutralizes the remaining tissue debris in the root canal system [8].

However, Ca(OH)2 cannot be considered as a universal intra-canal medicament, since it is not equally effective against all bacteria found in the root canal. Indeed, several studies have reported the failure of Ca(OH)2 to eliminate enterococci effectively as they tolerate high pH values, varying from 9 to 11 (9). Therefore, research for newer alternative intra-canal medicaments is necessary.

Chlorhexidinegluconate has been used as irrigant and intra-canal medicament in endodontics. Its antimicrobial effect is related to the cationic molecule binding to negatively charged bacterial cell walls, thereby altering cell's equilibrium and causing leakage of intracellular components. However, complete eradication of the bacteria is not guaranteed with the use of these agents and a search for a medicament that can predictably disinfect the root canal continues(10).

Natural products have been used in dental and medicinal practises for thousands of years and have become more popular today. **Ocimum sanctum** (Holy basil, Tulsi) is a plant native to India with known medicinal properties since the Vedic period. It is classified as a **"rasayana",** - an herb that nourishes a person's growth to perfect health and promotes long life. It has known antibacterial, antifungal and antiviral properties. The dried leaves of the plant can be powdered, mixed with mustard oil to make a dentrifice. It can prevent dental caries and apthous ulcers in the mouth . There are few studies documenting the use of herbal extracts as intra-canal medicaments (11).

Here in the present study the combination of Ca(OH)2 and chlorhexidine and herbal extract tulsi is used.

In this study, we are evaluating tulsi for its use as an intra canal medicament and comparing its antibacterial efficacy with the combination of calcium hydroxide and 1% chlorhexidine against *E faecalis* in both primary teeth and permanent teeth.

Aim:

To compare the efficacy of CAOH & CHX combination and tulsi in

primary teeth and permanent teeth against E.faecalis Till now many studies were done in primary and permanent teeth separately,in the present study there is comparision between primary and permanent teeth.

MATERIALS AND METHODS:

- Calcium hydroxide paste(CAL-EXCEL)
- 1% Chlorhexidine
- Tulsi extract (95%) material used.

TOOTH PREPARATION:

60 teeth which are 30 primary and 30 permanent teeth extracted due to infection or excessive caries that have radiographically visible no >1/3 physiological or pathological root resorption. Then, root surfaces were cleaned using a curette. Crowns of the teeth were removed to cement enamel junction and 60 roots which are 30 primary and 30 permanent obtained. An access cavity orifice was processed, and the pulpal tissue was removed by using a barbed broach and the root canals were enlarged to a size no.30 K file (blue number). In the course of instrumentation, all root canals were irrigated with 5% NaOCl at each file used. Afterwards initial preparation, the smear layer of each sample was removed in an ultrasonic bath with 17% ethylenediaminetetraacetic acid (pH 7.8) and 5% NaOCl for 10 min. After the roots were dried by using sterile paper points, the apical foramina were covered using flowable composite resin and the root canals were immersed in acrylic resin blocks , which allowed handling of the teeth during the experiment. The samples were autoclaved at 121°C.

Contamination of root canals:

E. faecalis(ATCC 29212) was used as the test organism cultured in Brain Heart Infusion broth (BHI). Bacterial inoculumwas standardized to 0.5 McFarland turbidity standards. Root canals were incubated at 37°C for 21days.

Disinfection procedures:

Group I : Saline (20 teeth with 10 primary and 10 permanent)
Group II : Calcium hydroxide and chlorhexidene combination(1:1) (20 teeth with 10 primary and 10 permanent)
Group III :Tulsi (20 teeth with 10 primary and 10 permanent)
The 60 samples were randomly separated into three experimental groups (n = 20) with 10 primary and 10 permanent teeth in each group. The twenty teeth are disinfected with calcium hydroxide and 1% chlorhexidine combination. Another group are disinfected with tulsi extract. One group as a control filled with saline (n = 20).

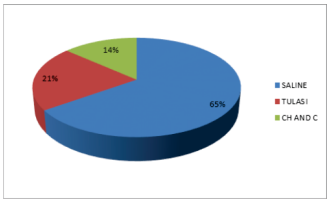
First sampling was taken after 24hrs of placement of intra canal medicament. Dentinal scrapings were collected from all root canals using H-files or sterile round bur& transferred into agar plates containing brain heart infusion broth which is the nutrient medium for growth of microbes. After this period microbial growth was measured by CFU/ml. After sampling the root canals were sealed with parafiin wax. This must be repeated on third day and seventh day.

STATISTICAL ANALYSES:

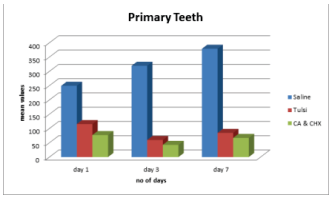
One way Anova,Two way Anova, T test and Multiple comparison post hoc tests are used for the statistical analysis.

RESULTS:

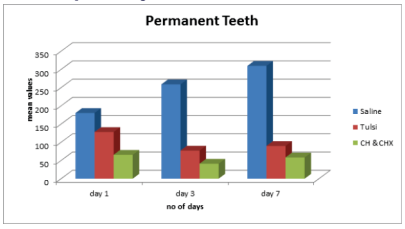
Graph 1 shows calcium hydroxide and Chlorhexidine is better efficient than tulsi in day 1, 3 and 7 in primary teeth. **Graph 2** shows calcium hydroxide and Chlorhexidine is better efficient than tulsi in day 1, 3 and 7 in permanent teeth.. It shows that CH and CHX is better in both primary and permanent teeth than tulsi. **Graph 3** gives the comparision of primary and permanent teeth and shows that tulsi is efficient in primary than permanent teeth.



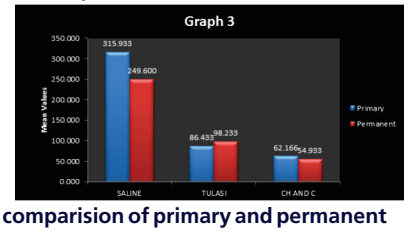
Calcium hydroxide and Chlorhexidine vs tulsi - SD value



Graph 1: calcium hydroxide and Chlorhexidine vs tulsi in day 1,3 and 7 in primary teeth.



Graph 2 :calcium hydroxide and Chlorhexidine vs tulsi in day 1,3 and 7 in permant teeth.



Graph : comparision of primary and permanent

Table 1: calcium hydroxide and Chlorhexidine vs tulsi in day 1,3 and 7 in primary and permanent teeth.

	DAYS	SALINE		TULASI		CH AND C	
		Mean	S.D	Mean	S.D	Mean	S.D
PRIMARY	Day 1	249.400a	5.929	115.300c	6.945	77.00c	5.395
	Day 3	319.400b	5.542	59.600a	5.542	42.600a	5.358
	Day 7	379.000c	5.099	84.400b	6.720	66.900b	5.744
	p value	0.000		0.000		0.000	
PERMANENT	Day 1	180.300a	6.945	128.000c	5.333	65.500c	3.894
	Day 3	258.900b	5.896	77.000a	5.228	41.100a	4.533
	Day 7	309.600c	5.125	89.700b	4.762	58.200b	3.553
	p value	0.000		0.000		0.000	

Table 2: calcium hydroxide and Chlorhexidine vs tulsi averagemean and SD primary and permanent teeth.

score	N	SALINE		TULASI		CH AND C		p value
		Mean	S.D	Mean	S.D	Mean	S.D	
Primary	30	315.933c	54.135	86.433b	23.991	62.166a	15.614	0.000
Permanent	30	249.600c	54.416	98.233b	22.593	54.933a	11.098	0.000
p value		0.000		0.000		0.000		

Table 3: calcium hydroxide and Chlorhexidine vs tulsi p value

	N	Mean	S.D
SALINE	60	282.766c	63.361
TULASI	60	92.333b	23.858
CH AND C	60	58.550a	13.917
p value		0.000	

DISCUSSION:

Biomechanical preparation alone is not capable of thoroughly eliminating microorganisms from the complex root canal system. Investigators have noted that bacteria in instrumented, unfilled canals can multiply and reach their pretreatment numbers in 2 to 4 days. In such cases, dressing of root canals using antimicrobial medicaments are advocated *E. faecalis*is a resistant microorganism that plays an important role in persistent periapical lesions (12).

This study was a modest attempt to evaluate and compare the antibacterial efficacy of calcium hydroxide paste and 1% chlorhexidine combination and tulsiextract in both primary and permanent root canals infected by *E. faecalis.*

Calcium hydroxide is believed to have many of the properties of an ideal root canal dressing mainly due to its alkaline pH Estrela et al., claimed that Ca(OH)2 by means of hydroxyl ions inhibits bacterial enzymes of the bacteria's cytoplasmic membrane, generating the antibacterial effect(13).

Chlorhexidinegluconate is a cationic bisguanide that seems to act by adsorbing onto the cell wall of the microorganisms and causing leakage of intracellular components. Chlorhexidine was used in gel formulation because it imparts important properties such as low toxicity to periapical tissues, viscosity that keeps the active agent in contact with the root canal walls and dentinal tubules (14, 15). In the present study 1% CHX gel is used.

A study conducted by Sonam Bhandari et al.,evaluated the antimicrobial efficacy of 2% chlorhexidine gel,propolis and calcium hydroxide against E.faecalis in permanent teeth and it states that 2% chlorhexidine consistently demonstrated significant inhibition at day 1 ,3 and 5.In present study calcium hydroxide and chlorhexidine combination got better inhibition when compared to tulsi at day 1 ,3 and 7 in both in primary and permanent teeth.(16).

AR Prabhakar et al.,compared the antibacterial efficacy of calcium hydroxide ,2% chlorhexidine gel and turmeric extract in permanent teeth and stated that 2% chlorhexidine gel had most effective antibacterial activity at day 1 ,3 and 7. In present study calcium hydroxide and chlorhexidine combination got better inhibition of E.faecalis when compared to tulsi extract in both primary and permanent teeth (17).

Sharifian et al.,compared the effectiveness of chlorhexidine and two calcium hydroxide formulations on E.faecalis in permanent teeth states that viscous 2% CHX and mixture of CH with distilled water or 2% CHX were all effective for disinfection of root canal at 1,3 and 7 days and in present study calcium hydroxide and chlorhexidine combination got better inhibition of E.faecalis when compared to tulsi extract in both primary and permanent teeth.(18).

Sinha et al.,evaluated used calcium hydroxide paste ,chlorhexidine gel and a combination of both as intracanal medicament in permanent teeth stated CH showed limited efficacy against facultative anaerobes and candidiaspp.but was effective against obligate anaerobes,whereas CHX and combination were effective against all tested microorganisms which is similar to present study ie.,CH and CHX combination got better result in both primary and permanent teeth.

Nageshwar Rao et al.,showed that paste made from calcium hydroxide and chlorhexidine was significantly more effective than that made from calcium hydroxide and saline agaistE.faecalis in permanent teeth which is similar to present study that combination of CH and CHX is better than tulsi in both permanent and primary teeth.(19).

The anti inflammatory action of essential oil extract of *Ocimum sanctum* is attributed to the presence of alpha linolenic acid. It is an omega-3 fatty acid which inhibits both lipoxygenase and cyclooxygenase pathways i.e. it has a dual inhibitory action. The alpha linolenic acid is progressively metabolized to 6, 9, 12, 15 octadecatetranoic acids, stearadonic acid and eicosapentaenoic acid, which are the end products. It was concluded that the local infiltration of dexamethasone produced a significant anti-inflammatory effect on the periapical tissues of teeth with vital or partially necrotic pulp tissue. However, steroidal preparations have local side effects such as immunosuppression and impairment of periapical healing(16). Hence the use of essential oil extract of *Ocimum sanctum* having a good anti-inflammatory property is advantageous over the other steroidal/non steroidalintracanal medicament(17).

An ex-vivo study N.Y Navin Mishra etaland an animal model study documented the antibacterial and anti- inflammatory properties of the essential oil extract of *Ocimum sanctum*, for its proposed use as an intra canal medicament. In the present study tulsi is also efficient in reducing e.faecalis count but comparatively less efficacy than calcium hydroxide and CHX combination, but in present study CH and CHX combination got better result when compared to tulsi in both primary and permanent teeth.

CONCLUSION:

Within the limitation of the study calcium hydroxide and chlorhexidine combination is more efficient than tulsi by the end of first, third and seventh day in both primary and permanent teeth. Tulsi is better in primary teeth than permanent teeth as the second better choice.

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