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

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 take over and provide a favourable environment for healing (5,6).

Enterococcus faecalis 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 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 tulsi is used.

Aim:
To compare the efficacy of CAOH & CHX combination and tulsi in both primary teeth and permanent teeth.

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 comparison post hoc tests are used for the statistical analysis.

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

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

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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 comparison 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. Afterward 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 inoculum was standardized to 0.5 McFarland turbidity standards. Root canals were incubated at 37°C for 21 days.

**Disinfection procedures:**
- Group I: Saline (20 teeth with 10 primary and 10 permanent)
- Group II: Calcium hydroxide and chlorhexidine combination (1:1)
- 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 24 hrs of placement of intra canal medicament. Dentinal scrapings were collected from all root canals using H-files or sterile round burr & 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 paraffin 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 comparison of primary and permanent teeth and shows that tulsi is efficient in primary than permanent teeth.

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

<table>
<thead>
<tr>
<th>DAYS</th>
<th>SALINE</th>
<th>TULASI</th>
<th>CH AND C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D</td>
<td>Mean</td>
</tr>
<tr>
<td>PRIMARY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>249.400a</td>
<td>5.929</td>
<td>115.300c</td>
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<tr>
<td>Day 3</td>
<td>319.400b</td>
<td>5.542</td>
<td>59.600a</td>
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<tr>
<td>Day 7</td>
<td>379.000c</td>
<td>5.099</td>
<td>84.400b</td>
</tr>
<tr>
<td>p value</td>
<td>0.000</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>PERMANENT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>180.300a</td>
<td>6.945</td>
<td>128.000c</td>
</tr>
<tr>
<td>Day 3</td>
<td>258.900b</td>
<td>5.896</td>
<td>77.000a</td>
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<tr>
<td>Day 7</td>
<td>309.600c</td>
<td>5.125</td>
<td>89.700b</td>
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<td>p value</td>
<td>0.000</td>
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**Table 2:** calcium hydroxide and Chlorhexidine vs tulsi average mean and SD primary and permanent teeth.

<table>
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<th>SCORE</th>
<th>N</th>
<th>SALINE</th>
<th>TULASI</th>
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<tr>
<td></td>
<td></td>
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<td>54.135</td>
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<td></td>
<td>62.166a</td>
<td>15.614</td>
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<tr>
<td>PERMANENT</td>
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<td>249.600c</td>
<td>54.416</td>
<td>98.233b</td>
<td>22.593</td>
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<td>11.098</td>
<td></td>
<td>0.000</td>
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<tr>
<td>p value</td>
<td>0.000</td>
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hydroxide and chlorhexidine was significantly more effective than calcium hydroxide alone against facultative anaerobes and candida spp. but was effective against obligate anaerobes, whereas CHX and combination were effective at low toxicity to periapical tissues, viscosity that keeps the active ingredient 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 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 candida spp. 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.(19).

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 against E. 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 lipoxigenase and cyclooxygenase pathways i.e. it has a dual inhibitory action. The alpha linolenic acid is progressively metabolized to 6, 9, 12, 15 octadecatetraenoic acids, stearidonic 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, steroid 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 steroidal antiinflammatory medicament (17).

An ex-vivo study N.Y Navin Mishra et aland 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 the 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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