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Research article

Chemical composition and antimicrobial efficacy of calcium hydroxide with peppermint oil and to compare its effect with calcium hydroxide with saline against root canal pathogens of deciduous teeth

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ABSTRACT

For endodontic treatment in deciduous teeth, thorough instrumentation and cleaning is required which may not be possible due to difficult anatomic configuration of deciduous teeth. So root canal filling material at least should possess antimicrobial efficacy. Therefore, present study was planned to find out the bioactive ingredients and antimicrobial efficacy of calcium hydroxide mixed with peppermint oil (CaOH+P) and its effect was compared with routinely used calcium hydroxide with saline (CaOH+S) in the field of pediatric dentistry. To find out the bioactive ingredients in peppermint oil, gas chromatography mass spectrophotometry was performed and for evaluating antimicrobial efficacy, agar diffusion method was employed in which, Muller Hincton agar was used. In MH agar plates, punching was done at two equidistant points and test materials were filled and kept in incubator at 37ºC for 24 hours. Zone of inhibition was measured in millimeter. Six times repetitions of the procedure were performed. Statistically data was analyzed by using ANOVA and Tukey's post-hoc comparison test. P-value <0.05 was used for level of significance.

It was observed from the results that bioactive ingredients in peppermint oil were octanol (48.17%) followed by menthol (20.45%) and other ingredients were very less in quantity. Zone of inhibition obtained in CaOH+P oil paste against root canal pathogens in decreasing order were against Staph.aureus>E.coli>E.faecalis=P.aeruginosa with statistically significant difference (0.0001, p<0.05) while in CaOH+S paste, antimicrobial efficacy in decreasing order was E.Coli>Staph.aureus=E.faecalis>P.aeruginosa which was not significant statistically (0.373, p>0.05)

Zone of inhibition obtained in CaOH+P oil paste for Staph.aureus were higher as compared to CaOH+S paste, while for other root canal pathogens i.e; E.coli, E.faecalis and P.aeruginosa, CaOH+S paste showed larger zones as compared to CaOH+P oil pastedisease

Keywords: Calcium hydroxide mixed with peppermint oil paste, calcium hydroxide mixed with saline paste, antimicrobial efficacy, gas chromatography mass spectrophotometry.

INTRODUCTION

Root canal treatment in deciduous teeth is challenging because the root canal configuration of deciduous molars shows that roots are very thin, tortuous and curved with ribbon like canals along with numerous accessory and lateral canals. Therefore, instrumentation and cleaning of root canals become difficult. There should be root canal filling material which can fill the accessory and lateral canals of deciduous molars and cause antimicrobial efficacy.

Calcium hydroxide has been used as root canal filling material in pediatric dentistry as root canal filling material for deciduous teeth because of its properties like biological properties associated with release of Ca^{+} ions and antibacterial properties associated with release of OH^{-} ions due to its high pH.

Various vehicles have also been tried. Out of which, use of calcium hydroxide mixed with saline or distilled water was more. But it said that with water-based vehicle, calcium hydroxide dissociates faster into Ca^{+} and OH^{-} ions and remains only for short duration of time for its action [1].

Therefore, in the present study, peppermint oil has been used as it is oily vehicle and also its known antimicrobial action available in the literature. Expecting that, after mixing calcium hydroxide with peppermint oil, it will remain in the area of action for longer duration to show its antimicrobial efficacy.

In the literature, menthol and cavacrol are the two main ingredients which have been thought to be in higher conc. in peppermint oil. So, to understand the composition of peppermint oil which was used for the present study, gas chromatography and mass spectrophotometry was done. Studies by use of peppermint oil have been found in medical literature. But use of peppermint oil is only available with respect to its use in mouthwash. Considering the beneficial properties associated with peppermint oil, present study was carried out to first to find out the composition of peppermint oil which is used for the present research work and secondly to find out the antimicrobial efficacy of calcium hydroxide mixed with peppermint oil against root canal pathogens of deciduous teeth and to compare its effect with the routinely used calcium hydroxide mixed with saline [2].

MATRIALS AND METHODS

Present study was an *in vitro* study. It was approved by institutional ethical committee. Peppermint oil which was used in the present study was procured from Aroma Tantra, Mumbai. Calcium hydroxide powder (Prevost Denpro Limited, Jammu, India) was mixed with peppermint oil and was compared with calcium hydroxide powder mixed with saline paste [3].

Gas Chromatography/ Mass Spectrometry (GC/MS)

200µl of peppermint oil was mixed in 10ml methanol. It was vortexed and allowed to stand for 5 minutes. Syringe of 0.45µm was filtered and pepprmint oil then injected on GC/MS for analysis.

Column used was of fused silica capillary column DB 5-MS (15m x 0.25mm i.d., 0.25um) with column temperature, 40^0 (2 min)-8^0C/min-150^0C (0 min), 10^0C/ minute-300 ^{0}C (10 minutes.

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RESULTS

The composition of peppermint oil obtained by using gas chromatography mass specrophotometry analysis. Total 26 ingredients were obtained in peppermint oil, out of which major conc. was that of octanol (48.17%) followed by menthol (20.45%) and D-Limonene (13.66%). Other ingredients which were less in conc. were Glutaconic acid (2.95%), Methanone (2%), Estragole (0.97%), Carenol (0.99%), Caranone (1.95%), Menthol acetate (2.59%), Anisic acid 4, dinitrophenyl ester (0.36%), Propanedioic acid (0.27%), P-Methane-3,8 did,cis 1,3, trans (0.27%), 4-Isopeny-1 methyl 1,2 cyclohexadiol (0.63%), Cyclohexanemethaniol,2 hydoxy, 4 trimethyl (0.76%), a boubonene (0.61%), P-Methane-3,8 did,cis 1,3, trans (1.30%), Caryophyllene (1.26%), Cubebene (0.28%), Mourolene (0.15%), Ylangene (0.75%), Cadinal (10) 4- diene (0.14%), 3- Carene (0.18%), Spiro(azetidin, 2-one 42 tricyclodecane) (0.11%), 2,6- Dimethyl 1,3,6 heptatriene (0.29%), Silabenzene-1- methyl (0.05%). Zones of inhibition in mm of CaOH+P paste against root canal pathogens. Zones of inhibition in mm for *Staph.aureus* were larger i.e; 25.66±0.51 followed by for *E.coli* in which it was 16.33±1.36 and equal zones of inhibition for *E.faecalis* and *P.Aeruginosa* i.e; 12.66±1.03 respectively with statistically significant difference (p-value: 0.0001, $p<0.05$).

Table 3 and Graph 2 shows zones of bacterial growth inhibition in mm of CaOH+S paste against root canal pathogens. Zones of inhibition shown by CaOH+S paste for *Staph.aureus* was 17.33±3.26, for *E.coli* it was 19.33±1.63, for *E.faecalis,* it was 17.33±3.01 and for *P.aeruginosa*, it was 17.00±3.52 respectively. One way analysis of variance showed that the difference was not statistically significant (0.373, $p>0.05$) between and within groups [6].

DISCUSSION

Success of endodontic treatment is dependent upon the reduction or elimination of the infecting bacteria. Complex anatomy of deciduous teeth prevents complete elimination of microorganisms from the root canals. Various materials as an intracanal antimicrobials have been used in dentistry.

Calcium hydroxide has been used for a variety of purposes since its introduction into dentistry in the early part of the twentieth century. Calcium hydroxide has a high pH and it is chiefly used in dentistry because of its ability to stimulate mineralization and antibacterial properties.

Peppermint oil and its constituents have been found to be used commercially in food, cosmetics and pharmaceutical industries. Menthol is used in the form of raw material in toothpaste, toothpowder, chewing gums, mouth fresheners, candies, confectionary, cough drops, analgesic balms and perfumes. The fresh or dried leaves are the source

of mint and are used in breath fresheners, antiseptic mouth rinses, toothpaste, chewing gum, mint chocolate teas, drinks, beverages, jellies, syrups, candies, ice creams. Menthol is the substance which is responsible to give the peppermint oil their characteristic aromas and flavors.

The antimicrobial activity of peppermint oil is due to the presence of terpenoides menthol, menthone, 1-8-cineole, methyl acetate, menthofuran, isomenthone, limonene, b-pinene, germacerene-d, trans-sabinene hydrate and pulegone. In the present study, 26 ingredients were obtained by GS/MS analysis in peppermint oil. Octanol was found to be in higher conc. (48.17%) followed by menthol (20.45%) and D-Limonene (13.66%). Other ingredients were very less in conc. which included Glutaconic acid, Methanone, Estragole, Carenol, Caranone, Menthol acetate, Anisic acid 4, dinitrophenyl ester, Propanedioic acid, P- [7].

CONCLUSION

CaOH+P paste is oily based material. Calcium hydroxide also has its own advantages in terms of antimicrobial effect and mineralization. When calcium hydroxide is mixed with peppermint oil with combined effect of both the ingredients, antimicrobial efficacy shown will be beneficial against the root canal pathogens of deciduous teeth.

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