Original Article

Comparative evaluation of antimicrobial efficacy of calcium hydroxide, Himalayan pink salt as an intracanal medicament against *Enterococcus faecalis*: An *in vitro* study

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ABSTRACT

Context: Microorganisms play an important role in the etiology of pulp and periapical diseases. For successful endodontic treatment, their control and elimination is important. The microorganisms may remain after root canal preparation intracanal medicament help in reducing remaining microorganism and can provide a favorable environment for periapical tissue repair. The increase in side effects and safety concerns of conventional medicaments has led to the recent popularity of herbal alternative medications.

Aims: The purpose of this study was to investigate and compare the effectiveness of calcium hydroxide and Himalayan pink salt for the elimination of *Enterococcus faecalis* bacteria in extracted teeth samples.

Materials and Method: Sixty extracted single-rooted human permanent teeth randomly divided into four groups. Group 1 – Control ($n = 15$); Group 2 – Ca $(OH)_2$ ($n = 15$), Group 3 Himalayan Pink Salt ($n = 15$), and Group 4 – Ca $(OH)_2$ and Himalayan pink salt ($n = 15$). The intracanal medicaments were placed in teeth specimen infected with *E. faecalis* incubated in the anaerobic condition for 37 C. Dentine shavings were collected from the specimens at different time interval at the 1st, 3rd, and 5th day and planted on agar plates. Colony-forming units (CFUs) were further be counted.

Statistical Analysis Used: The data were statistically analyzed with the one-way analysis of variance, followed by Scheffe's multiple comparisons means to check the differences in CFU count between the groups ($P < 0.05$).

Results: The present study showed that Himalayan pink salt exerted antibacterial activity in combination with calcium hydroxide.

Keywords: Antimicrobial efficacy, *Enterococcus faecalis*, Himalayan pink salt, intracanal medicament, root canal disinfection

INTRODUCTION

The role of microorganisms in the etiology of pulp and periapical diseases has been emphasized in the dental literature over the period of time. Their control and elimination is necessary for the successful endodontic treatment, which is a challenging task owing to the complex anatomy of the root canals.

Although the bacterial reduction is achieved predominantly through irrigation and intra-canal medicaments, partly through shaping of the canals. After the appointment bacterial counts usually decrease, but leaving the canal empty between appointments leads to bacterial counts to original levels.[1,2] Therefore, intracanal medication is important as it can complement the work of chemomechanical instrumentation and to reduce the counts of remaining bacteria, thus supporting the healing of periapical tissues.[3]

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Several chemicals and therapeutic agents are used to disinfect the root canal. $Ca(OH)_2$ is the gold standard of intracanal medicament. $Ca(OH)_2$ has its antimicrobial property due to its high pH; it destroys and alters the bacterial polysaccharides in the cell wall. Enterococci which can tolerate high pH values, varying from 9 to 11 making it difficult to eliminate from the canal with the use of $Ca(OH)_2$.[4] Therefore, research for newer alternative intracanal medicaments is necessary.

There has been an increase in antibiotic misuse and resistance of microorganisms to them. However, the disadvantages associated with chemical intracanal medicaments, their abuse or overuse and misuse leading to toxicity, and cytotoxic reaction, herbal and natural products have become more popular as they are associated with low toxicity and fewer side effects when compared to chemical medicaments.[5]

Natural products have been used in dental and medicinal practices for thousands of years have become more popular today. Natural remedies are increasingly finding their way into endodontic treatment with agents such as *Morinda citrofolia*, *Triphala*, curcumin, and propolis being evaluated as irrigants and intracanal medicaments.

Since ancient era, salt has been used as preservative because of its antimicrobial property. Himalayan pink salt is basically a rock salt mined from Kherwa district of Punjab region of Pakistan. The salt often has a pinkish tint due to mineral impurities. It is most commonly used as a food additive, also as a decorative lamps, and spa treatments material for cooking and food preservation.[6]

Enterococcus faecalis is the most commonly used organism in the microbiological evaluation of newer intracanal medicament. Keeping these above concepts in mind, to date, there is no reported research on the dentinal tubule disinfection by Himalayan pink salt against *E. faecalis*. Hence, the aim of the present study is to explore a new material, Himalayan pink salt as an intracanal medicament and comparing its antibacterial efficacy with the commonly used medicaments, i.e., calcium hydroxide against *E. faecalis*.

MATERIALS AND METHOD

This *in vitro* study was conducted at the Department of Conservative Dentistry and Endodontics of dental college and was approved by the Institutional Ethical Committee. The chunks of Himalayan pink salt were collected from the local distributor, and it was crushed and then sieved to obtain coarse powder and a uniform texture with the help of mortar and pestle. Calcium hydroxide was used in the powder form as supplied by the manufacturer.

Preparation of dentine specimen

Sixty single-rooted permanent human teeth freshly extracted for orthodontic reasons and fully developed root apices were collected from department of oral and maxillofacial surgery. The samples were stored in normal saline during the study.

The model by Haapasalo and Orstavik was modified as human teeth were used and cementum was kept intact.[7] Teeth were disinfected with 5.25% sodium hypochlorite (NaOCl). Calculus and tissue remnants were removed using ultrasonic scaler. Only teeth with single root and canal were selected for the study. The Weine's type I canal configuration was confirmed by using digital radiograph in mesiodistal and buccolingual planes.

Teeth were decoronated below the cement enamel junction and the apical part of the root to obtain 6 mm of middle section of the root by using a diamond disc. The internal diameter of the root canals was standardize with Gates Glidden drill no. 2 (Mani Inc., Tochigi, Japan).

The specimens were placed in an ultrasonic bath of 17% EDTA (AvuPrep, DENTAL AVENUE PVT. LTD, Thane, Maharashtra, India) 5 min followed by 3% NaOCl (PHOTON-RPC PVT. LTD.) for 5 min to remove organic and inorganic debris. The traces of chemicals, which was left behind, were removed by immersing the dentin specimens in an ultrasonic bath containing distilled water for 5 min. All the specimens were sterilized in an autoclave at 121°C for 15 min.

Contamination of specimen

The *E. faecalis* (ATCC 29212) was used in the study. The pure culture of freeze dried bacteria was suspended in 5 ml of Brain – Heart Infusion broth (EOS Laboratories, Thane, Maharashtra, India) followed by incubation for 24 h at 37°C according to manufacturer instruction. Each dentin block was placed in presterilized microcentrifuge tubes containing 1 mL of the BHI broth and was inoculated with 10 μL inoculums of bacteria using micropipette. These samples were incubated for a period of 21 days at 37°C in incubator. At the end of 48 h, samples were transferred into fresh broth containing *E. faecalis*. This whole procedure was carried out under laminar flow machine (Thermo Fisher Scientific Inc., Waltham, MA USA) to prevent any contamination.

Antimicrobial assessment

After the contamination of the specimen at the end of 21 days, using 5 mL of sterile saline specimen were irrigated to remove the incubation broth. The samples were sequentially assigned to the following groups ($n = 15$):

- Group 1: Saline (control group)
- Group 2: Calcium hydroxide (1:1) (PREVEST DenPro)

- Group 3: Himalayan pink salt
- Group 4: Calcium hydroxide and Himalayan pink salt. (1:1).

The respective medicaments were placed in the root canal, and with the use of paraffin wax, the apical end of the specimen was sealed; the specimens were then incubated in an anaerobic environment for 37°C. Microbial cells assessment was carried out at the end of 1, 3, and 5 days of incubation, with five specimens at each intervals of time. Harvesting of dentin was carried out preparing the root canal circumferentially using sterile Gates Glidden drills no. 3 and no. 4, respectively, in slow speed hand piece. With the help of paper point, the dentinal shavings were collected from the tip of the drills. Then, the shavings were then transferred into 1 ml of sterile broth and incubated in the anaerobic environment for 24 h. After 24 h, the contents were serially diluted with 100 µL of broth in 100 µL of sterile saline five times. About 50 µL of the dilution was then plated on BHI agar plates and incubated for 24 h at 37°C. Digital colony counter was used to count the colonies of the test organism, and readings were tabulated.

RESULTS

Statistical analysis

The data were statistically analyzed with the one-way analysis of variance, followed by Scheffe's multiple comparisons means to check the differences in colony-forming units (CFUs) count between the groups ($P < 0.05$). The analysis was performed with the Statistical Package for the Social Sciences software (SPSS version 16.0, SPSS Inc., Chicago, IL, USA).

RESULTS

The present study showed that Himalayan pink salt exerted antibacterial activity.

Pairwise comparison of CFU shows significant result on all the three intervals.

Group 4: combination of $Ca(OH)_2$ and Himalayan pink salt was found to be the most effective against *E. faecalis* on all days of incubation, as shown in Table 1.Intergroup comparison between the groups showed significant difference between $Ca(OH)_2$, Himalayan pink salt and combination of $Ca(OH)_2$ and Himalayan pink salt on all the days [Table 2].

DISCUSSION

The favorable outcome of endodontic treatment depends upon the effective control of root canal infection. Various limitations such as anatomical complexity and difficulty in accessing the root canal by instrument and irrigant render the problem for complete disinfection of the root canal. It is believed that residual microorganisms can be further reduced by dressing the canal with an intracanal medicament.

The *in vitro* model proposed by Haapasalo M and Orstavik D has been used to study the efficacy of intracanal medications in the disinfection of dentinal tubules. This model has clear limitations because it does not reflect the situation in apical dentin, which is mostly sclerotic.[8] To compensate for the apical sclerosis, the middle third of the canal was selected.

E. faecalis is Gram-positive cocci that occur singly, in pairs or short chains[9,10] can survive harsh environments such as extreme alkaline pH (9.6) and a temperature of 60°C for 30 min.[11] It has certain virulence factors such as lytic enzymes, cytolysin, pheromones, and lipotechoic acid, which suppresses the action of lymphocytes, potentially contributing to endodontic failure.[12] In addition, it is very small to invade and live within dentinal tubules and can endure prolonged periods of starvation.[13] When nutritional supply becomes available, it can utilize serum as a nutritional source that originates from alveolar bone and periodontal ligament.[14] *E. faecalis* passively maintains pH homeostasis by a proton pump. It has been shown to synthesize a variety of stress proteins when exposed to adverse environmental conditions.[15]

Various intracanal medicaments have been used to minimize or eradicate *E. faecalis* from the root canal space but have not been very successfully efficient.[16-18] Several *in vitro* and *in vivo* studies have tested herbal extracts for better antimicrobial activity and biocompatibility.[17,19,20] Considering the resistance of *E. faecalis*, there is a pressing need to investigate intracanal medicament that can totally eliminate it.

The present study compared Himalayan pink salt and calcium hydroxide for antimicrobial efficacy against *E. faecalis*.

Calcium hydroxide believed to have many of the properties of an ideal root canal dressing mainly due to its alkaline pH.[21] $Ca(OH)_2$ shows its antibacterial effect by leaching hydroxyl ion, which can act on bacteria's cytoplasmic membrane by inhibiting the bacterial enzyme.[22]

In this study, calcium hydroxide showed minimal antimicrobial effect compared with Himalayan pink salt. For an intracanal dressing to act effectively, it is necessary to occupy the entire pulp space thereby having close proximity with root canal microbiota. $Ca(OH)_2$ behaves similarly. Indeed such

Table 1: Pairwise comparison of colony-forming units

Pair	Saline-Ca $(OH)_2$	Saline-Himalayan pink salt	Saline-combination	Ca $(OH)_2$-Himalayan pink salt	Ca $(OH)_2$-combination	Himalayan pink salt-combination
Mean difference						
At day 1	441.00	401.60	300.20	39.40	140.80	101.40
P	0.001*	0.001*	0.001*	0.633	0.003*	0.031*
At days 3	439.00	373.20	381.80	65.80	57.20	8.60
P	0.001*	0.001*	0.001*	0.003*	0.009*	0.942
At days 5	446.40	375.80	395.80	70.60	50.60	20.00
P	0.001*	0.001*	0.001*	0.001*	0.001*	0.290

Post hoc tukey test; * indicates significant at $P \leq 0.05$

Table 2: Intergroup comparison of mean change

	Day 1-days 3 (%)	Day 1-days 5 (%)	Days 3-days 5 (%)
Saline	0.65	0.00	0.65
$Ca(OH)_2$	1.46	4.94	3.53
pH salt	16.67	17.34	0.57
Combination	34.05	38.21	67.27

contact does not occur in the total root canal system, where microorganisms can be located inside the dentinal tubules.

Moreover, the reason for this limited antibacterial effect of calcium hydroxide on *E. faecalis* could be attributed to the dentin buffering effect. In order to have antibacterial effect within dentinal tubules, the ionic diffusion of $Ca(OH)_2$ should surpass the dentin buffer capacity, reaching pH levels sufficient enough to destroy bacterial strains, particularly *E. faecalis* which can survive a high pH of 11.5.[23] Another factor is the location of bacteria within dentinal tubules or enclosed in anatomical variations along with this the arrangement of the *E. faecalis* colonizing the root canal walls, which can reduce the antibacterial effects of calcium hydroxide, since the cells situated at the periphery of colonies can shield those present at a much deeper level inside the tubules.[24]

According to the results of the present study, Himalayan pink salt has better antibacterial efficacy when compared to combination on day 1, but there was no significant difference between them on day 3 and day 5.

The introduction of environmental stresses such as extreme temperature, pH, hypo-osmotic or hyperosmotic pressure, the depletion of nutrients, and the use of preservatives[25] has an adverse effect on the physiology of microbial cells, leading to a reduction in growth rate or cell death. Hypertonic salt solution induces the cell death[26] and reduces the cohesion of biofilm matrices.[27,28]

The present study shows that, Himalayan pink salt can be considered as potential antimicrobial intracanal medicament.

The Himalayan pink salt contains more calcium, iron, magnesium, and potassium than regular salt.[29] The potassium said to be have the effective antimicrobial effect on microorganism.

However, the combination of Ca $(OH)_2$ and Himalayan pink salt shows the most effective antimicrobial efficacy on the 1st, 3rd, and 5th day. The possible reason could be the synergistic or the additive effect.

Further research on the use of these alternative medicaments must be considered before its application in clinical practice.

CONCLUSION

Within the limitations of this study, it can be concluded that Himalayan pink salt can be used as a potential intracanal medicament in combination with $Ca(OH)_2$.

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Conflicts of interest
There are no conflicts of interest.

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