ORIGINAL ARTICLE

Mustard gel is an effective intracanal medicament against Enterococcus faecalis: an in-vitro study on extracted teeth

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ABSTRACT

Background and Objective: Root canal treatment is hampered by the presence of resilient bacteria within the canals of teeth which leads to the need for incorporation of intra-canal medication. This study aimed to assess the effectiveness of mustard gel against the highly resistant *Enterococcus faecalis* (*E. faecalis*) bacteria in comparison with calcium hydroxide ($Ca(OH)_2$) paste as an intra-canal medicament.

Methods: This experimental study comprised forty extracted single-rooted teeth which were equally divided into two groups. In group 1, $Ca(OH)_2$ paste was applied whereas in group 2, mustard gel was used. After disinfection, each tooth was sectioned horizontally into three equal parts. The standardized middle section was inoculated with a controlled strain of *E. faecalis* followed by the application of intra-canal medicaments for a week in both groups. The bacterial colonies were counted as colony-forming units (CFU/ml). Data were entered and analyzed by using Statistical Package for Social Sciences version 25.0.

Results: Microscopically, *E. faecalis* appeared as cocci-round shaped in chains or pairs. The median bacterial count was 1,550 (775-2,500) for group 1 ($Ca(OH)_2$ paste) as compared to only 400 (200-775) in group 2 (mustard gel) ($p < 0.001$).

Conclusion: Mustard gel can be used as a cost-effective, readily available, and safe herbal alternative to $Ca(OH)_2$ paste for root canal treatments.

Keywords: *Enterococcus faecalis*, root canal therapy, calcium hydroxide, mustard gel, medicament.

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Introduction

Infections associated with dental caries typically manifest as endodontic infections, which are treated through a procedure known as root canal therapy. A traditional root canal treatment involves disinfection of the root canals, followed by preparation and shaping of the canals. After that, the root canal is filled and sealed to maintain the tooth's natural structure.[1] However, despite meticulous adherence to root canal treatment protocols, several factors can contribute to the failure of the treatment itself including discrepancies in the anatomical configuration, reinfection due to resilient bacteria, a failure to identify canals and incomplete obturation.[2] Primary endodontic infections are associated with *Enterococcus faecalis* (*E. faecalis*), a Gram-positive facultative anaerobic cocci nine times more frequently in root canal treatment failures.[3]

Endodontist suggests the use of irrigants and intra-canal medicament with residual anti-microbial action to prevent root canal re-infection and successful endodontic treatment.[4] A wide variety of intra-canal medications including calcium hydroxide, ($Ca(OH)_2$) poly antibiotic pastes, and phenolic compounds (formocresol and glutaraldehyde) are used to prevent re-infection.[5] Despite all these medications, $Ca(OH)_2$ is still considered a gold standard, conventional, and effective bactericidal compound however, both *Candida albicans* and *E. faecelis* are extremely resistant to it.[6,7]

It is important to develop new antimicrobial agents to combat the rising threat of antibiotic resistance to public health.[8] Medicinal plants are considered to be the most effective alternative by the World Health Organization, thus the purpose of searching for new agents is to find out the plants containing bioactive therapeutic agents.[9]

In dentistry, some of the natural products used medically are Azadirachta *indica* (neem), *Aloe vera*, Miswak, and *Propolis* (honey).[10] The combination of mustard (*Brassica* species) and honey is used as an intra-canal remedy in one local case study yet it has not been studied alone as an intra-canal medicament.[11] Due to the drawbacks of $Ca(OH)_2$ (ineffective against *E. faecalis*), there was a need to find a new alternative intra-canal medicament. Considering all the medical and oral uses of mustard, the present study aimed to evaluate the anti-bacterial efficacy of mustard gel against *E. faecalis* and compared it with conventional medicament of $Ca(OH)_2$ paste.

Methods

This experimental study was conducted in the Surgery Department of de' Montmorency College of Dentistry, Lahore, and the Microbiology Department of General Hospital, Lahore, Pakistan, during 6 months after approval from the Institutional Review Board. A convenient sampling technique was used. A total of forty non-carious, single-rooted permanent mandibular premolars, as well as maxillary and mandibular anterior teeth (central incisors and lateral incisors), with closed apex and single canal which had been previously extracted for orthodontic or periodontal reasons, were included and randomly divided into two groups using lottery method. $Ca(OH)_2$ paste was applied to 20 samples in group 1, while the mustard gel was applied to other 20 samples in group 2. While carious, restored, cracked, fractured, and permanent multirooted teeth with multiple canals were excluded. Apart from these, teeth with developmental anomalies were not part of the study.

Freshly extracted single-rooted teeth were preserved in 0.9% sterile normal saline for two hours. Any soft tissue present on the tooth surface was removed manually by using a 5.25% sodium hypochlorite (NaOCl) solution. Following this, the coronal, middle, and apical sections of each tooth were horizontally prepared using a carbide disc fitted with a straight handpiece by maintaining a uniform reference at the cement-enamel junction to maintain consistency. Being the wider and straighter part of the root canal, the middle segment of 5 mm was selected for further analysis which was enlarged to standardize the internal diameter. Subsequently, the samples underwent a process of immersion in a 17% Ethylenediamine tetra-acetic acid solution followed by a 5.25% NaOCl solution for 5 minutes to eliminate the smear layer. Finally, each tooth sample was individually packaged in a sterilization pouch and subjected to autoclaving at 121°C for 30 minutes at 15 psi to ensure sterilization.[12]

Black mustard seeds (Brassica *nigra*), weighing 227 grams procured from a local market and authorized by a Taxonomist, Botany Department at Government College University Lahore, Pakistan (Voucher # GC.Herb.Bot.3698) were finely ground to the powder using a blender. The black mustard seeds were immersed in acetone within disposable conical flasks within a clean, dry soxhlet extractor with a mass/volume ratio of 1:2. The solvent, containing 30% mustard extract, was meticulously collected after 96 hours, while the insoluble fraction persisted within the thimble. A Whatman No. 1 filter paper was used to generate a transparent and clear filtrate.[13] $Ca(OH)_2$ paste was formed after mixing $Ca(OH)_2$ powder (1.5 mg) in sterile saline (1 ml) in a ratio (1.5:1). Prabhakar et al.[14] Fresh stock culture plates frequently prepared from de man-rogosa-sharpe (MRS) agar were stored in a refrigerator at −20°C using the standard strain of *E. faecalis* (M.0181 ATCC # 14506). Sinha, et al.[15] Bacterial suspension was obtained by incubating the broth at 37°C for 48 hours after extracting one colony of *E. faecalis* from the MRS agar plate to broth.

Dentinal tubules were infected by immersing segments of teeth in 2 ml of MRS broth containing the *E. faecalis* culture followed by incubation separately in Eppendorf tubes for 5 days at 37°C. Following the incubation period, the segments were carefully removed from the broth using sterile forceps, rinsed with 5 ml of sterile water, and dried using sterile gauze.

The prepared specimens were positioned upright and gently pressed into nutrient agar dishes. Medicaments were applied to the canals of teeth of both groups using a lentulospiral to ensure thorough packing of the medicaments. These Petri dishes were then placed back in the incubator at 37°C and maintained at 100% humidity for 1 week. After 1 week, the tooth segments from the Petri dishes were removed and thoroughly irrigated with 2 ml of distilled water before being dried with gauze and paper points. Then, dentinal shavings from the canals were collected using separate spoon excavators which were spread by sterilized disposable loop 10 ul (0.01ml) on MRS plates to test for bacterial suspensions.[16] The plates were then incubated at 37°C for 48 hours. *Enterococcus faecalis* colonies were identified by positive Gram staining[17] under a light microscope with 100X magnification with the assistance of a Microbiologist. Bacterial colonies were expressed as colony-forming units/ml (CFU/ml), a reliable technique for determining the efficacy of antibacterial agents in reducing viable bacteria.[18,19]

Statistical analysis

Statistical Package for Social Sciences version 25.0 was used to analyze the data. Mean ± Standard Deviation and median with interquartile range were determined for numerical variables (bacterial count CFU/ml). Shapiro-Wilk test was used to test the normality of data. Mann Whitney U test was applied to compare the bacterial count (CFU/ml) between the two groups. A *p*-value of ≤0.05 was considered as significant.

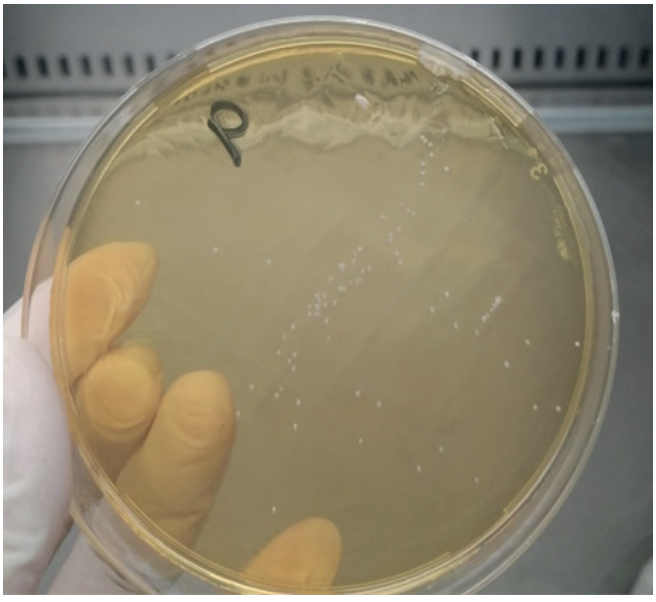

Figure 1. *Enterococcus faecalis growth colonies (CFU) on MRS agar plate.*

Results

Bacterial colonies were counted visually on plates as shown in Figure 1.

Microscopically, *E. faecalis* appeared as cocci-round shaped in chains or pairs, and the bacterial count collected was multiplied by 100 to get the count per milliliter (CFU/ml) (Figure 2).

The bacterial count was not normally distributed as assessed by applying the Shapiro-Wilk test as presented in Table 1.

The median bacterial count was 1,550 (775-2,500) for group 1 while it was 400 (200-775) for group 2 (Table 2). Mann Whitney U test was used to compare the bacterial count between the two groups as given in Table 2 and there was a significant difference in median bacterial count between the two groups ($p < 0.001$).

Discussion

The primary goal of endodontic treatment is to achieve complete disinfection of the root canal, including both the processes of canal preparation and obturation. In spite of the rigorous protocols used during root canal therapy, there is always the possibility of reinfection within the root canal system. Consequently, various intra-canal medications are suggested to reduce this risk of re-infection[20,21]. The main objective of the study was to assess the effectiveness of mustard gel in comparison to $Ca(OH)_2$ paste as intra-canal medicaments against *E. Faecalis*.

An *in-vitro* study conducted on extracted third molars assessed the effectiveness of chloramine T and $Ca(OH)_2$ as intra-canal medications in conjunction with NaOCl irrigation.

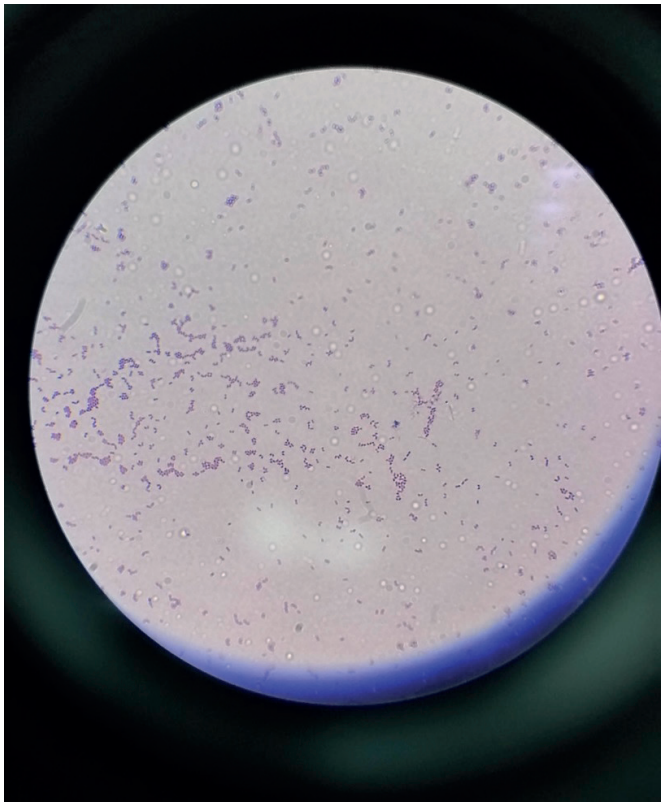

Figure 2. *Photomicrograph shows the chains and pairs of cocci-round-shaped bacterial colonies on Gram staining.*

Table 1. *Distribution of bacterial count in both groups.*

Group	Shapiro-Wilk		
	Statistic	Df	*p*-value
Calcium hydrooxide (Group 1)	0.841	20	**0.004**
Mustard gel (Group 2)	0.446	20	**0.000**

*Shapiro-Wilk.

$Ca(OH)_2$ was found to be ineffective against these resistant strains when administered intracanally.[13] In a similar study, Lakhani et al.[20] used different intra-canal medicaments, including saline, 2% chlorhexidine (CHX) gel, $Ca(OH)_2$, triple antibiotic paste, and moxifloxacin, and assessed the bacterial load at 1, 7, and 10-day intervals using extracted single-rooted teeth. He reported $Ca(OH)_2$ ineffective against *E. faecalis*, whereas CHX proved to be significantly effective.[20] Ghabraei et al. [21] used 2% CHX gel in combination with Ca(OH)2 against *E. faecalis* and found this combination much more effective in eliminating bacteria from the canals in a shorter period of time as compared to $Ca(OH)_2$ alone.[21]

Recent research has placed significant emphasis on exploring herbal alternatives for dental applications. Numerous studies have highlighted the therapeutic benefits of natural herbal extracts in dental practice, showcasing their

Table 1. *Comparison of bacterial count between both groups.*

Group	Bacterial count (CFU/ml)				
	Mean ± SD	Median (Inter-quartile range)	Min.	Max.	*p*-value#
$Ca(OH)_2$ group 1	1,785 ± 1,363	1,550 (775-2,500)	300	6,200	< 0.001*
Mustard gel group 2	855 ± 1639	400 (200-775)	000	7,600	

#Mann Whitney *U* test.
*Significant.

antibacterial, antifungal, anti-inflammatory, analgesic, and anesthetic properties.[22,23] These herbal extracts have been extensively tested against various pathogens in the root canal[6].

Kaur and colleagues conducted a comparative study using conventional oral medications, such as NaOCl and CHX, with herbal remedies, specifically *A. indica* and *Aloe* barbadensis. NaOCl showed the greatest reduction in microbial counts, followed by *A. indica* and CHX gel. Thus, highlighting the utilization of herbal medicaments as an effective alternative to conventional medicaments due to their potent antimicrobial properties. [5]

In the current study, the focus was on evaluating mustard gel as an intra-canal medicament, being a novel approach as it has not been studied earlier in inter-appointment medication during root canal treatment. Previously, mustard oils and extracts have been studied primarily for their ability to inhibit bacterial growth.[24, 25] Locally, a single *in-vitro* study was conducted on the mixture of mustard oil and honey on infected root canals with necrosed pulps. Camphorated paramonochlorophenol and a mixture of honey and mustard oil were applied, and the minimum inhibitory concentration was calculated against isolated bacterial species, with *E. faecalis*, *Staphylococcus aureus*, *Streptococcus*, and *Prevotella melaninogenicus* being the most frequently identified bacteria and a higher antibacterial efficacy for the mixture of honey and mustard oil was observed in that study.[11] While comparing to the previous study, the mustard gel was found more effective than $Ca(OH)_2$. The results of the present study supported the use of mustard gel as a new herbal alternative medicament during primary and secondary root canal treatment with the advantages of easy accessibility, cost viability, more shelf life, lesser toxicity, and no reported resistance so far. However, further *in vitro* studies are necessary to fully understand the clinical implications of mustard gel and its medicaments in endodontics.

Conclusion

The mustard gel is a cost-effective, readily available, and safe herbal alternative to conventional root canal medicaments with the potential to successfully mitigate the harmful effects of resilient bacteria. Clinical trials may however further confirm the findings of this study.

Limitations of the Study

The limitation of an *in-vitro* study relates to predicting the clinical efficacy of the tested medicaments. A large sample size could be taken for future studies. Further randomized clinical trials are recommended to evaluate the antibacterial efficacy of mustard gel as an intra-canal medicament in human dental practice.

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List of Abbreviations

$Ca(OH)_2$	Calcium hydroxide
CFU	Colony forming unit
E. faecalis	*Enterococcus faecalis*
NaOCl	Sodium hypochlorite

Conflict of interest

None to declare.

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Ethical approval

Ethical approval was granted by the Institutional Ethics Committee of Post Graduate Medical Institute/Ameer-ud-Din Medical College, Lahore, Pakistan, vide Letter No. 00/185-20 dated 06-10-2020.

Authors' contributions

AZ: Acquisition and analysis of data, drafting of the manuscript.
SN: Concept and design of the study, critical intellectual input.
MM: Acquisition and analysis of data.
All authors approved the final version of the manuscript to be published.

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