

Mustard Gel versus Chlorhexidine Gel as Root-Canal Medicament Against *Enterococcus Faecalis*: An *in vitro* Study

Ambreen Zahra¹, Shazia Naz², Mashal Mazhar³, Fatima Saeed⁴, Bilal Ahmed Khalid¹ and Asdaq Hussain⁵

¹Department of Operative Dentistry, Avicenna Medical and Dental College, Lahore, Pakistan

²Department of Operative Dentistry, de'Montmorency College of Dentistry, Lahore, Pakistan

³Department of Operative Dentistry, University of Lahore, Lahore, Pakistan

⁴Department of Orthodontics, de'Montmorency College of Dentistry, Lahore, Pakistan

⁵Department of Conservative Dentistry and Endodontics, National Medical College, Birgunj, Nepal

ABSTRACT

Objective: To evaluate the efficacy of mustard gel versus chlorhexidine (CHX) gel in treating the most resistant *Enterococcus faecalis* (*E. faecalis*) bacteria as intra-canal medications.

Study Design: Randomised controlled trial (*in vitro* study).

Place and Duration of the Study: Department of Operative Dentistry, de'Montmorency College of Dentistry, in collaboration with Lahore General Hospital, between March and October 2021.

Methodology: Forty single-rooted teeth, extracted and randomly allocated, were divided into two groups. Group 1 underwent treatment with CHX gel, while Group 2 was treated with Mustard gel. Subsequently, after disinfection, each tooth was horizontally divided into three equal parts. The middle section was standardised and inoculated with a controlled strain of *E. faecalis*. Intra-canal medications were administered for seven days. Bacterial colonies were quantified as colony forming units (CFU/mL) and subjected to analysis using Statistical Package for Social Sciences (SPSS) version 25.

Results: The median bacterial count observed was 1,550 (range 775 - 2,500) for Group 1, contrasting with 400 (range 200 - 775) for Group 2, indicating a significant difference in bacterial count between the groups ($p < 0.001$).

Conclusion: Mustard gel demonstrated significantly superior efficacy compared to chlorhexidine gel against *E. faecalis* in root canal procedures.

Key Words: Chlorhexidine, *Enterococcus faecalis*, Mustard, Root canal medicament, Antimicrobial drug-resistance, Root canal irrigants.

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INTRODUCTION

Root canal therapy, alternatively termed endodontic treatment, entails the cleaning and shaping of the root canal system, followed by the placement of a sterile material in the pulpal space. Endodontic treatment is a commonly performed procedure in dentistry.¹ However, the success of both initial treatment and any necessary retreatment depends on various factors such as the presence of peri-radicular infection, residual necrotic pulp tissue, broken instruments, perforations, inadequate filling of the root canals, missed canals, and others.²

Endodontists recommend using irrigants and intra-canal medications (ICM) with antimicrobial properties to prevent reinfection of the root canal and ensure successful treatment.³ The primary goal of ICM is to eradicate organisms present in the pulp space. However, organisms residing in dentinal tubules cannot be eradicated solely through chemo-mechanical preparation. Properly dressing the root canal with antiseptic medicaments during treatment intervals is crucial to prevent an increase in residual bacteria.^{4,5}

Calcium hydroxide (Ca(OH)₂) stands as a traditional, widely accepted, and efficacious bactericidal agent.⁶ Nonetheless, both *Candida albicans* and *E. faecalis* exhibit a high resistance to it. Notably, *E. faecalis* emerges as a prominent contributor to failures in root canal treatment. This emphasises the necessity for heightened vigilance in managing infectious conditions within root canals.⁷ Chlorhexidine (CHX) was found to be effective against resistant microbes.⁸ However, it has reported adverse reactions (ADRs) including dry mouth, taste alteration, hypogeusia, mouth and tongue numbness, oral and tongue pain and ulceration, subjective discolouration, calculus buildup, and external tooth discolouration with prolonged usage. Less

Correspondence to: Dr. Ambreen Zahra, Department of Operative Dentistry, Avicenna Medical and Dental College, Lahore, Pakistan
E-mail: drambreen514@gmail.com

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frequent adverse effects include parotitis, oral tingling, discomfort of the tongue, and shredding of the oral mucous membrane. Additionally, a growing concern linked to CHX use is the emergence of antimicrobial resistance (AMR), presenting a significant threat. Even minimum CHX exposure can result in cross-resistance to antibiotics. This necessitated the identification of an alternative ICM with minimal side effects.⁹

With pathogenic bacteria showing increased resistance to antibiotics and chemotherapeutic substances, scientists are turning their attention towards alternative products and therapies for the treatment of oral ailments. Herbal products have better biocompatibility with the body, strong antimicrobial, antioxidant and anti-inflammatory effects, accessibility, and minimal side effects. As per the World Health Organization (WHO), herbal medicine comprises plant-derived preparations or materials. Although herbs hold promise as replacements for traditional treatments for oral health problems, there is still a gap in the understanding regarding their effects on the oral cavity, way of action, and adverse reactions.¹⁰ In endodontics, herbs are mainly used for root canal disinfection, especially in response to the undesired effects linked with many synthetic root-canal medications. The rationale of the current research was to concentrate on irrigation with herbal alternatives in endodontic practice.¹¹ Mustard seeds (*Brassica nigra*) play an important role due to its medicinal value. *Brassica nigra* seeds have been demonstrated to impart effect against bacterial and fungal infections and inflammatory conditions.¹² Nevertheless, it has not been investigated as an isolated ICM. Thus, the objective of the current study was to evaluate mustard gel as an alternative ICM to CHX gel against *E. faecalis*.

METHODOLOGY

This randomised controlled trial took place at the Operative Dentistry Department, de'Montmorency College of Dentistry, affiliated with the Microbiology Department of Lahore General Hospital, spanning from March to October 2021. Approval for the study was granted by the Institutional Review Board (IRB) of Lahore General Hospital (Synopsis No: 00-185-20).

The sample size was calculated using WHO calculator version 12.2.6 by the following formula keeping the confidence level equal to 95% and power of study equal to 90% with 5% margin of error:

$$n = \frac{(\sigma_1 + \sigma_2)^2 \times (Z_{1-\alpha/2} + Z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$$

Where $Z_{1-\alpha/2}$ = standard level of significance = 95% = 1.96, $Z_{1-\beta}$ = power of test = 90% = 1.28.

μ_1 = case mean bacterial count (CFU/ml) of the control group = 2.48, μ_2 = mean bacterial count (CFU/ml) experimental group = of 1.22, δ_1 = control (0.1303) Variance = (standard deviation)², and δ_2 = experimental (0.1643). The relevant study considered the sample size in each group was 20.

The present study included forty teeth that were healthy and free from caries. The inclusion criteria included permanent

lower non-carious, premolars, maxillary and mandibular anterior teeth (central and lateral incisors) possessing a solitary root, single canal, and sealed apex. These teeth were initially slated for extraction due to orthodontic or periodontal considerations. A convenience sampling approach was employed, and using the lottery method the selected teeth were randomly allocated into two groups; Group 1 comprised 20 samples treated with CHX gel, while Group 2 consisted of 20 samples treated with mustard gel. Exclusion criteria included teeth with caries, restorations, cracks, fractures, and multiple roots or canals, as well as those exhibiting developmental anomalies.

Following the extraction, the tooth samples were stored in 0.9% sterile saline for two hours. Any residue on the tooth surface was cleaned using a 5.25% sodium hypochlorite (NaOCl) solution. Three equal sections of each tooth were horizontally prepared using a carbide disc. The middle 5mm segment was selected and enlarged to standardise the internal diameter. To eliminate the smear layer, the study samples were dipped in 17% ethylene diamine tetra-acetic acid (EDTA) solution followed by a 5.25% sodium hypochlorite (NaOCl) solution for 5 minutes. Each tooth sample was autoclaved at 121°C for 30 minutes at 15 psi to achieve sterilisation within an individualised sterilisation pouch.

Black mustard seeds were ground to a powder and extracted using acetone. The mustard extract was mixed with gelling material carboxy methyl cellulose to make gel.¹³ From 20% CHX solution, 2% CHX solution was made. The 2% CHX gel was formulated by blending 2% CHX gluconate solution (Product # 0219136090) and 8 grams of hydroxyethyl cellulose (1% natrosol). The pH was maintained at 7 by pH metre, and a magnetic stirrer was used during the formulation of gel.¹⁴ Fresh stock culture plates of *E. faecalis* were prepared and stored in a refrigerator. Bacterial suspension was obtained from *E. faecalis* culture, and tooth segments were immersed in MRS (de Man, Rogosa, and Sharpe) broth containing the bacterial culture followed by incubation.

After incubation, the segments were removed, rinsed, and dried. Medicaments were carefully packed into the tooth canals of teeth in both groups to ensure complete packing. Petri dishes containing the specimens were then placed in the incubator at 37°C for one week. Afterwards, tooth segments were removed, irrigated, and dried, and dentinal shavings were collected and spread on MRS plates.¹⁵ *E. faecalis* colonies were identified, and bacterial colonies were expressed as colony-forming units per millilitre (CFU/mL).¹⁶

After the study was completed, the Statistical Package for Social Sciences (SPSS) version 25.0 was utilised to assess the data. For numerical variables (bacterial count CFU/mL), a median with an interquartile range was given. The data's normality was evaluated by Shapiro-Wilk's test. The Mann-Whitney U test was used to compare the bacterial count (CFU/mL) between the two groups. A p-value of ≤ 0.05 was considered statistically significant.

Table I: Comparison of bacterial count among groups.

Group	Bacterial count (CFU/ml)		Min.	Max.	p-value
	Mean ± SD	Median (Interquartile range)			
CHX gel	2100 ± 1456	1950 (700 - 3150)	400	5200	<0.001*
Mustard gel	855 ± 1639	400 (200 - 775)	000	7600	

*Mann-Whitney U test.

RESULTS

The median bacterial count of the CHX gel group was 1950 (700 - 3150), and the median bacterial count of the mustard gel group was 400 (200 - 775). Mann-Whitney U test showed that there was a notable difference in median bacterial count among the two groups ($p < 0.001$, Table I).

DISCUSSION

The present study evaluated the antibacterial efficacy of CHX gel and mustard gel as ICM against *E. faecalis*. The result of the study showed that the bacterial count (CFU/mL) of the mustard gel group was lower as compared to CHX gel. A notable difference was observed between the two groups. This finding is consistent with another study that found herbal extracts as effective antibacterial agents.¹⁷

Previously the antibacterial effects of mustard oils and extracts have been investigated. In Pakistan, a single study has been performed which constitutes mustard oil and honey applied in combination inside infected root canals. The minimum inhibitory concentration was calculated against test strains including *E. faecalis*, a mixture of honey and mustard oil was found to have significant antibacterial efficacy.¹⁸ Similar result was found in the present study favouring the antibacterial efficacy of mustard as an ICM. However, the present study is different in context of the methodology used and the bacterial count tests.

Herbal alternatives have found popularity nowadays in the medicinal field. Several herbal medicaments have been used so far including *propolis*, *triphala*, *neem*, and *miswak*. The herbal alternatives are found to be more effective medicaments with no toxic effect.¹⁷ A similar *in vitro* study was carried out by Mahendra *et al.*, in which nisin, *neem*, platelet-rich plasma, and *propolis* were assessed as ICM against *E. faecalis*. The methodology was similar to the current research. The extracted tooth specimens were inoculated with the test strains of *E. faecalis*. CFU of herbal medicaments were calculated to be minimal. The results supported the use of herbal alternatives.¹⁹ However, the current research studied a new herbal medicament i.e. mustard gel.

A comparable study assessed the antimicrobial effectiveness of *Septilin*, Ca(OH)_2 , and 2% CHX against test strains of *E. faecalis* and *Streptococcus mutans*. The study concluded that *Septilin*, Ca(OH)_2 , and CHX exhibited similar antibacterial efficacy against both *E. faecalis* and *Streptococcus mutans*. However, unlike the present study, the evaluation of the medicament's antibacterial effectiveness was conducted using the agar diffusion test instead of colony counts.²⁰

A study evaluated the antimicrobial efficacy of various combinations of ICM, including 2% CHX gluconate with Ca(OH)_2 , clindamycin hydrochloride 5% with Ca(OH)_2 , a combination of glycerin 60% and Ca(OH)_2 , and pure glycerin 100%, against *E. faecalis*. The Ca(OH)_2 with 2% CHX gluconate combination showed a notable reduction in bacterial counts. In contrast, the other three samples exhibited a slight increase in the number of *E. faecalis*.²¹ The present study did not analyse the combinations of medicaments. Also, its rationale was to evaluate an alternative herbal ICM.

Likewise, an Indonesian study assessed the effectiveness of various root canal medicaments, namely CaOH_2 combined with 2% CHX digluconate and CaOH_2 combined with 25% *propolis*, against *E. faecalis* bacteria. The results demonstrated that the combination of Ca(OH)_2 and 25% *propolis* exhibited the highest antibacterial effect compared to another group.²²

The present study has some limitations. Firstly, being *in vitro* in nature, it faced the challenge of accurately assessing the clinical effectiveness of the experimental components. The result of the medicaments was dependent on many factors such as the properties of the materials used, the formulation method of gel, and the extraction protocol. The characteristics of the culture medium and the protocol to transfer the samples may also influence the results. Additionally, future studies may require a larger sample size.

CONCLUSION

Mustard gel demonstrated significantly superior efficacy compared to chlorhexidine gel against *E. faecalis* in root canal procedures. Mustard gel emerges as an economical, easily accessible, and secure botanical substitute to traditional root canal medications, exhibiting promise in combating the adverse impacts of stubborn bacteria. However, additional clinical trials are warranted to validate the outcomes of this research.

ETHICAL APPROVAL:

The study was conducted after obtaining approval from the Institutional Review Board (IRB) of Lahore General Hospital.

PATIENTS' CONSENT:

Written and informed consent was taken from the patient before the initiation of the study.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

AZ: Manuscript writing.

SN: Proofreading.

MM: Discussion writing.

FS: Literature search.

BAK: Editing.

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