

Comparative Evaluation Of The Effect Of Intracanal Medicaments Calcium Hydroxide Paste, Chlorhexidine Gluconate Gel, And Aloe Vera Gel—On Root Dentin Microhardness: An In Vitro Study

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Abstract

Background

Intracanal medicaments are routinely used during endodontic therapy to eliminate residual microorganisms and prevent reinfection. However, prolonged exposure may alter the physical properties of root dentin, particularly its microhardness. Therefore, evaluating their effect on dentin microhardness is essential for preserving the structural integrity of endodontically treated teeth.

Aim

To comparatively evaluate the effect of calcium hydroxide paste, chlorhexidine gluconate gel, and aloe vera gel on the microhardness of root canal dentin after 7 days of intracanal medicament application.

Materials and Methods

This in vitro experimental study was conducted on 32 extracted human single-rooted permanent teeth. Following decoronation, standardized root specimens were prepared and the canals were instrumented using the ProTaper rotary file system. The specimens were randomly allocated into four groups (n = 8): Group I—Saline (control), Group II—Calcium hydroxide paste, Group III—Chlorhexidine gluconate gel, and Group IV—Aloe vera gel. The respective intracanal medicaments were placed using a Lentulo spiral, and the specimens were stored for 7 days. Subsequently, the medicaments were removed, and the middle third of each root was sectioned longitudinally to obtain 3-mm-thick specimens. Dentin microhardness was evaluated using a Vickers microhardness tester. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value of ≤ 0.001 was considered statistically significant.

Results

The control group demonstrated the highest mean dentin microhardness (69.79 ± 0.61 VHN), followed by the Aloe vera gel group (67.69 ± 0.34 VHN), the chlorhexidine gluconate gel group (65.10 ± 1.20 VHN), and the calcium hydroxide group (53.85 ± 0.82 VHN). One-way ANOVA revealed a highly significant difference among the study groups ($F = 622.77$, $p \leq 0.001$). Tukey's post hoc analysis demonstrated statistically significant differences between all pairwise group comparisons ($p \leq 0.001$). Among the experimental medicaments, Aloe vera gel preserved dentin microhardness most effectively, whereas calcium hydroxide produced the greatest reduction in dentin microhardness.

Conclusion

Within the limitations of this in vitro study, all intracanal medicaments influenced root dentin microhardness after 7 days of application. Aloe vera gel exhibited the least adverse effect on dentin microhardness among the tested medicaments, while calcium hydroxide caused the greatest reduction. These findings suggest that Aloe vera gel may be considered a promising biocompatible intracanal medicament for preserving the mechanical properties of root dentin, although further long-term clinical studies are required to validate its clinical applicability.

Keywords: Aloe vera, Calcium hydroxide, Chlorhexidine gluconate, Dentin microhardness, Intracanal medicament

Introduction

Successful endodontic treatment depends on effective elimination of microorganisms from the root canal system and prevention of reinfection. Although biomechanical preparation plays a crucial role in reducing the microbial load, complete disinfection of the root canal cannot be achieved by instrumentation alone because of the complex internal anatomy of the root canal system, including fins, isthmuses, lateral canals, and dentinal tubules, which serve as reservoirs for persistent microorganisms. Therefore, intracanal medicaments are routinely employed as an adjunct to chemomechanical preparation to enhance microbial elimination and improve the outcome of endodontic therapy.^[1]

An ideal intracanal medicament should possess broad-spectrum antimicrobial activity, prolonged antibacterial effect, excellent biocompatibility, ease of placement and removal, and should preserve the structural integrity of root dentin. In addition to eliminating residual microorganisms, intracanal medicaments should not adversely affect the physical and mechanical properties of dentin, as these properties are essential for maintaining the long-term strength and function of endodontically treated teeth.^[2]

Root dentin is a highly mineralized connective tissue composed of approximately 70% inorganic material, 20% organic matrix, and 10% water by weight. The hardness of dentin is largely dependent on its mineral content and serves as an important indicator of its mechanical integrity. Dentin microhardness reflects the resistance of dentin to localized deformation and is commonly used to evaluate changes in the mineral content of dentin following exposure to chemical agents or intracanal medicaments.^[2] A reduction in dentin microhardness may predispose teeth to crack

formation, decreased fracture resistance, and eventual treatment failure.^[3]

Among the available intracanal medicaments, calcium hydroxide has long been regarded as the gold standard because of its strong antimicrobial activity, high alkalinity, ability to neutralize bacterial endotoxins, and capacity to stimulate hard tissue formation. Its antibacterial effect is primarily attributed to the release of hydroxyl ions, which create a highly alkaline environment that is unfavorable for bacterial survival. Despite these advantages, prolonged application of calcium hydroxide has been reported to alter the organic matrix of dentin, resulting in reduced microhardness and diminished fracture resistance.^[4]

Chlorhexidine gluconate has gained considerable popularity as an alternative intracanal medicament because of its broad-spectrum antimicrobial activity, substantivity, and effectiveness against resistant microorganisms such as *Enterococcus faecalis*. Unlike calcium hydroxide, chlorhexidine maintains prolonged antimicrobial activity through adsorption onto dentin surfaces and gradual release over time. Although chlorhexidine is considered less aggressive toward dentin than calcium hydroxide, studies have reported varying effects on dentin microhardness depending on its concentration, formulation, and duration of application.^[5]

The increasing demand for biologically compatible and naturally derived therapeutic agents has stimulated interest in herbal intracanal medicaments. Herbal products offer several advantages, including lower toxicity, better biocompatibility, reduced incidence of adverse reactions, and multiple pharmacological properties. Consequently, they have emerged as promising alternatives to conventional

intracanal medicaments in contemporary endodontic practice.^[6]

Aloe vera (*Aloe barbadensis* Miller) is one of the most extensively investigated medicinal plants because of its antimicrobial, anti-inflammatory, antioxidant, immunomodulatory, and wound-healing properties. The therapeutic effects of Aloe vera are attributed to its rich composition of bioactive compounds, including anthraquinones, flavonoids, polysaccharides, vitamins, enzymes, amino acids, and minerals. Previous studies have demonstrated that Aloe vera exhibits antibacterial activity against several endodontic pathogens while maintaining excellent biocompatibility, making it a potential intracanal medicament.^[7]

In recent years, researchers have increasingly focused on evaluating not only the antimicrobial efficacy of intracanal medicaments but also their influence on the mechanical properties of root dentin. Since endodontically treated teeth are inherently more susceptible to fracture due to the loss of tooth structure and alterations in dentin composition, preservation of dentin microhardness has become an important consideration in selecting an intracanal medicament.^[8]

Several in vitro studies have compared the effects of calcium hydroxide, chlorhexidine, and various herbal medicaments on dentin microhardness. While calcium hydroxide consistently demonstrates excellent antimicrobial efficacy, numerous investigations have reported significant reductions in dentin microhardness following its prolonged application.^[2] Chlorhexidine generally exhibits a comparatively moderate effect on dentin hardness, whereas herbal medicaments such as Aloe vera have shown encouraging results in preserving dentin structure while providing satisfactory antimicrobial activity. However, the available evidence remains inconclusive because of variations in study design, specimen preparation, medicament concentration, duration of application, and testing methodology.^[9]

The Vickers microhardness test is widely accepted as a reliable and reproducible method for assessing the hardness of dental tissues. It measures the resistance of dentin to indentation under a standardized load and provides quantitative information regarding alterations in the mineralized structure of dentin following exposure to various chemical agents. Consequently, Vickers microhardness testing has

become one of the most commonly employed methods for evaluating the influence of intracanal medicaments on dentin properties.^[10]

Despite the widespread use of calcium hydroxide and chlorhexidine as intracanal medicaments, concerns remain regarding their potential effects on dentin integrity. At the same time, increasing evidence supporting the therapeutic potential of herbal alternatives such as Aloe vera necessitates further investigation to establish their effectiveness in preserving dentin microhardness while providing adequate antimicrobial action.^[11]

Comparative evaluation of conventional and herbal intracanal medicaments is essential for identifying agents that can achieve effective root canal disinfection without compromising the mechanical properties of dentin.^[12] Such investigations contribute to evidence-based clinical decision-making and may facilitate the development of safer and more biologically compatible endodontic treatment protocols.^[13]

Therefore, the present in vitro study was undertaken to comparatively evaluate the effect of calcium hydroxide paste, chlorhexidine gluconate gel, and Aloe vera gel on the microhardness of root canal dentin using the Vickers microhardness test. The findings of this study may provide valuable information regarding the influence of these commonly used intracanal medicaments on dentin integrity and assist clinicians in selecting medicaments that effectively balance antimicrobial efficacy with preservation of the mechanical properties of root dentin.

Materials And Methodology

Study Design

The present study was designed as an in vitro experimental study to comparatively evaluate the effect of calcium hydroxide paste, chlorhexidine gluconate gel, and Aloe vera gel on the microhardness of root dentin following intracanal medicament application for a period of seven days.

Study Setting

The study was carried out in the Department of Conservative Dentistry and Endodontics after obtaining approval from the Institutional Ethics Committee. All laboratory procedures were performed

under standardized conditions to ensure reproducibility and minimize experimental bias.

Sample Size Determination

The sample size was calculated using G*Power software with an alpha error probability (α) of 0.05, a statistical power ($1-\beta$) of 0.80, and four study groups. Based on an effect size (f) of 0.59, the minimum required sample size was calculated as 32 specimens. Accordingly, thirty-two extracted human single-rooted permanent teeth were included in the study and equally distributed into four groups, with eight specimens in each group.

Sample Selection

Thirty-two freshly extracted human single-rooted permanent teeth with completely formed apices were selected for the study. Following extraction, all adherent soft tissue debris and calculus were removed using an ultrasonic scaler. The teeth were cleaned thoroughly and stored in normal saline until use to prevent dehydration.

Inclusion Criteria

1. Extracted human single-rooted permanent teeth.
2. Teeth with fully developed roots and completely formed apices.
3. Teeth with a single straight root canal.
4. Teeth free from caries, restorations, cracks, fractures, internal resorption, and external resorption.

Exclusion Criteria

1. Teeth with more than one canal.
2. Teeth with root caries or developmental defects.
3. Teeth with previous endodontic treatment.
4. Teeth exhibiting root fractures, cracks, calcified canals, or severe root curvature.

Materials Used

The following materials and instruments were used in the study:

1. Extracted human single-rooted permanent teeth
2. ProTaper rotary file system
3. Calcium hydroxide paste
4. Chlorhexidine gluconate gel
5. Aloe vera gel
6. Normal saline
7. 17% EDTA gel
8. 3% Sodium hypochlorite solution

9. Lentulo spiral
10. Temporary restorative material
11. Vickers microhardness tester
12. Diamond disc
13. Digital Vernier caliper
14. Acrylic resin blocks
15. Low-speed handpiece
16. Distilled water

Specimen Preparation

The crowns of all selected teeth were removed at the cemento-enamel junction using a diamond disc under continuous water cooling to obtain standardized root specimens. The working length was established by introducing a size 10 K-file into the canal until its tip became visible at the apical foramen and subtracting 1 mm from this length.

Root canals were prepared using the ProTaper rotary file system according to the manufacturer's instructions until the desired apical preparation was achieved. During instrumentation, the canals were irrigated with 3% sodium hypochlorite solution after each instrument. Following completion of canal preparation, the smear layer was removed using 17% EDTA for one minute, followed by a final rinse with normal saline. The canals were dried thoroughly using sterile paper points.

Group Allocation

The prepared specimens were randomly assigned into four experimental groups ($n = 8$).

Group I: Control (Normal saline)

Group II: Calcium hydroxide paste

Group III: Chlorhexidine gluconate gel

Group IV: Aloe vera gel

Placement of Intracanal Medicaments

The respective intracanal medicaments were introduced into the prepared root canals using a Lentulo spiral to ensure uniform distribution throughout the canal space. In the control group, the canals were filled with normal saline.

Following placement of the medicaments, the access openings were sealed with a temporary restorative material to prevent contamination and leakage. The specimens were then stored under standardized laboratory conditions for seven days.

Removal of Intracanal Medicaments

After the experimental period of seven days, the temporary restoration was removed and the intracanal medicaments were flushed out using normal saline. The canals were irrigated thoroughly and dried with sterile paper points before specimen sectioning.

Preparation of Specimens for Microhardness Testing

Each root specimen was sectioned longitudinally using a diamond disc under continuous water cooling. A 3-mm-thick section from the middle third of the root was obtained for microhardness evaluation. The prepared sections were embedded in acrylic resin blocks with the dentin surface exposed.

The exposed dentin surfaces were polished sequentially using silicon carbide abrasive papers of increasing grit size under water irrigation to obtain a smooth, flat surface suitable for indentation testing.

Evaluation of Dentin Microhardness

Dentin microhardness was assessed using a Vickers microhardness tester. A predetermined load was applied to the dentin surface through a diamond indenter for a fixed dwell time according to the manufacturer's recommendations. Three indentations were made on each specimen at standardized locations in the middle-third radicular dentin, maintaining adequate spacing between adjacent indentations and from the canal wall to prevent overlapping stress fields.

The lengths of the indentation diagonals were measured using the integrated microscope, and the corresponding Vickers Hardness Number (VHN) was calculated automatically by the testing machine. The mean Vickers hardness value obtained from the three indentations was considered the final microhardness value for each specimen.

Statistical Analysis

The collected data were analyzed using the Statistical Package for the Social Sciences (SPSS) software (IBM SPSS Statistics, Version 26.0, IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean $\pm$ standard deviation.

The obtained data were subjected to statistical analysis using one-way Analysis of Variance (ANOVA),

followed by Tukey's post-hoc test for multiple pairwise comparisons. A p-value of ≤ 0.05 was considered statistically significant.

Observations And Results

The present in vitro study was done to evaluate the effect of different intracanal medicaments—calcium hydroxide pastes, chlorhexidine gluconate gel, and Aloe vera gel—on the microhardness of root canal dentin.

Intergroup Comparison of Mean Microhardness Values

The mean Vickers microhardness values of the four experimental groups are presented in Table 1 and illustrated in Graph 1.

The control group exhibited the highest mean dentin microhardness value (69.79 ± 0.61 VHN), indicating that saline did not alter the mechanical properties of root dentin. Among the experimental medicaments, the Aloe vera gel group demonstrated the highest mean microhardness value (67.69 ± 0.34 VHN), suggesting minimal alteration of dentin structure. The chlorhexidine gluconate gel group showed an intermediate mean microhardness value (65.10 ± 1.20 VHN), indicating a moderate reduction in dentin hardness. In contrast, the calcium hydroxide group recorded the lowest mean microhardness value (53.85 ± 0.82 VHN), demonstrating the greatest reduction in dentin microhardness among all the groups.

One-way ANOVA revealed a highly statistically significant difference in mean microhardness values among the four groups ($F = 622.77$, $p \leq 0.001$), indicating that the type of intracanal medicament significantly influenced the microhardness of root canal dentin.

Pairwise Comparison of Microhardness Values

To determine the differences between individual groups, Tukey's post-hoc multiple comparison test was performed, and the results are presented in Table 2.

The control group demonstrated significantly higher dentin microhardness than all experimental groups. Compared with Aloe vera gel, the mean difference was 2.10 VHN ($p \leq 0.001$). When compared with chlorhexidine gluconate gel, the mean difference increased to 4.69 VHN ($p \leq 0.001$). The largest difference was observed between the control group

and calcium hydroxide, with a mean difference of 15.94 VHN ($p \leq 0.001$), indicating a substantial reduction in dentin hardness following calcium hydroxide application.

Comparison between the Aloe vera gel and chlorhexidine gluconate gel groups revealed a statistically significant mean difference of 2.59 VHN ($p \leq 0.001$), demonstrating that Aloe vera preserved dentin microhardness significantly better than chlorhexidine gluconate gel. Likewise, Aloe vera gel showed significantly greater dentin microhardness than calcium hydroxide, with a mean difference of 13.84 VHN ($p \leq 0.001$), highlighting its superior ability to maintain dentin integrity.

The chlorhexidine gluconate gel group also demonstrated significantly higher dentin microhardness than the calcium hydroxide group, with a mean difference of 11.25 VHN ($p \leq 0.001$). These findings indicate that although chlorhexidine reduced dentin microhardness, its adverse effect was considerably less pronounced than that produced by calcium hydroxide.

Discussion

The primary objective of endodontic treatment is the complete elimination of microorganisms from the root canal system while preserving the structural integrity of the tooth. Although chemomechanical preparation substantially reduces the microbial load, complete disinfection of the complex root canal anatomy cannot be achieved by instrumentation and irrigation alone. Consequently, intracanal medicaments are placed between appointments to eliminate residual microorganisms, prevent reinfection, and improve the long-term prognosis of endodontic therapy. However, in addition to their antimicrobial efficacy, these medicaments should preserve the physical and mechanical properties of root dentin, particularly dentin microhardness, which plays a critical role in maintaining fracture resistance and the longevity of endodontically treated teeth. Therefore, the present study comparatively evaluated the effect of calcium hydroxide paste, chlorhexidine gluconate gel, and Aloe vera gel on the microhardness of root canal dentin.^[14]

The selection of calcium hydroxide paste, chlorhexidine gluconate gel, and Aloe vera gel in the present investigation was based on their established

antimicrobial properties and their widespread use or potential application as intracanal medicaments. Calcium hydroxide has traditionally been regarded as the gold standard intracanal medicament because of its broad-spectrum antimicrobial activity and ability to neutralize bacterial endotoxins.^[13] Chlorhexidine gluconate is well recognized for its substantivity and effectiveness against resistant microorganisms such as *Enterococcus faecalis*, whereas Aloe vera has recently gained attention as a herbal alternative because of its antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. These characteristics justified their comparison with respect to their influence on dentin microhardness.^[15]

The intracanal medicaments were maintained inside the root canals for a period of seven days. This duration was selected based on previous investigations demonstrating that one week provides sufficient time for intracanal medicaments to exert their maximum antimicrobial effect while reflecting the interval commonly adopted in clinical endodontic practice.^[3] Alsubait et al. reported that intracanal medicaments may be placed for periods ranging from one week to several months depending on the clinical situation, while the American Association of Endodontists recommends a minimum duration of one week in regenerative endodontic procedures to achieve adequate therapeutic efficacy.^[11] Likewise, Javidi et al. demonstrated that calcium hydroxide exhibited significantly greater antibacterial activity after seven days than after one day, and Mathew et al. reported that 2% chlorhexidine gel produced greater antimicrobial inhibition on the seventh day than on the second day. Similarly, Varshini et al. observed that Aloe vera exhibited maximum antimicrobial activity against *Enterococcus faecalis* after seven days of intracanal application. Therefore, a seven-day duration was considered appropriate for evaluating the influence of these medicaments on dentin microhardness.^[15]

The middle third of the root was selected for specimen preparation because it provides a relatively uniform dentin thickness and minimizes anatomical variations that may influence hardness measurements. A standardized dentin disc thickness of 3 mm was maintained in all specimens to ensure adequate mechanical stability during grinding, polishing, and indentation procedures. Grazziotin-Soares et al. reported that dentin specimens thinner than 2 mm may

undergo flexure or crack formation during testing, thereby affecting the accuracy and reproducibility of Vickers hardness measurements. Standardization of specimen thickness therefore contributed to obtaining reliable and comparable results among all experimental groups.^[16]

Before microhardness evaluation, the dentin specimens were sequentially ground and polished to obtain a flat and smooth testing surface. Surface preparation is essential because the Vickers hardness test requires a uniform polished surface for accurate indentation and precise measurement of the diagonal lengths. Previous studies by Fuentes *et al.* emphasized that hardness measurements should be performed on the polished dentin surface, whereas the opposite surface is generally embedded in acrylic resin or another mounting material for stabilization. The methodology adopted in the present study was therefore consistent with established protocols for dentin microhardness evaluation.^[17]

The Vickers microhardness test was selected because it is considered one of the most reliable and sensitive methods for evaluating changes in the mineralized structure of dentin. The test measures the resistance of dentin to localized plastic deformation and provides quantitative information regarding alterations in its inorganic and organic components following exposure to different intracanal medicaments. Consequently, Vickers hardness testing has become the preferred method for assessing the influence of endodontic materials on dentin mechanical properties.^[9]

The results of the present study demonstrated that all three intracanal medicaments produced a reduction in dentin microhardness when compared with the control group. However, the magnitude of reduction differed significantly among the medicaments. Calcium hydroxide produced the greatest reduction in dentin microhardness, chlorhexidine gluconate gel caused a moderate reduction, whereas Aloe vera gel exhibited the least reduction and maintained microhardness values closest to those of the control group. Statistical analysis confirmed highly significant differences among all experimental groups, indicating that the type of intracanal medicament significantly influences the mechanical properties of root dentin.^[15]

The marked reduction in dentin microhardness observed in the calcium hydroxide group may be attributed to its highly alkaline nature and its

interaction with the organic matrix of dentin. Purva Kadu *et al.* reported that the high pH and fine particle size of calcium hydroxide facilitate penetration into dentinal collagen fibrils, resulting in alteration of the three-dimensional structure of tropocollagen and a subsequent reduction in dentin microhardness and elastic modulus. Similarly, Yoldas *et al.* suggested that the prolonged alkaline environment created by calcium hydroxide induces proteolytic degradation of collagen and weakens the interaction between collagen fibers and hydroxyapatite crystals, thereby compromising the structural integrity of dentin. These mechanisms may explain the significant reduction in microhardness observed in the present study.^[17]

Chlorhexidine gluconate gel demonstrated a significantly smaller reduction in dentin microhardness than calcium hydroxide but a greater reduction than Aloe vera gel. This finding is consistent with previous reports indicating that although chlorhexidine possesses excellent antimicrobial activity and substantivity, prolonged contact with dentin may produce chemical interactions that influence its mechanical properties. Prabhakar *et al.* reported that 2% chlorhexidine causes a time-dependent reduction in dentin microhardness due to disruption of the interaction between collagen fibrils and hydroxyapatite crystals. Likewise, Ferraz *et al.* observed that chlorhexidine gel produced a cleaner and smoother root canal surface, suggesting mild softening of root dentin. These observations support the moderate reduction in microhardness observed in the chlorhexidine group of the present investigation.^[18]

Among the tested intracanal medicaments, Aloe vera gel exhibited the least reduction in dentin microhardness, indicating superior preservation of dentin structure. This beneficial effect may be attributed to the presence of several biologically active compounds, including anthraquinones, flavonoids, polysaccharides, vitamins, enzymes, amino acids, and minerals. In addition to its antimicrobial activity, Aloe vera has been shown to inhibit matrix metalloproteinases, particularly MMP-2 and MMP-9, thereby reducing collagen degradation and preserving the organic matrix of dentin. The ability of Aloe vera to maintain dentin integrity while providing antimicrobial action makes it a promising herbal alternative to conventional intracanal medicaments.^[19]

The control group, in which saline was used as the intracanal dressing, demonstrated the highest microhardness values because saline is biologically inert and does not chemically interact with dentin. Therefore, it preserved the natural mineral composition and mechanical properties of root dentin throughout the experimental period.^[5] The present findings indicate that although all intracanal medicaments influence dentin microhardness to some extent, the magnitude of this effect depends on the chemical characteristics of the medicament used.^[2]

Within the limitations of this in vitro investigation, Aloe vera gel demonstrated the greatest ability to preserve root dentin microhardness while maintaining the advantages of an intracanal medicament. Calcium hydroxide, despite its established antimicrobial efficacy, produced the greatest reduction in dentin microhardness, whereas chlorhexidine gluconate gel exhibited an intermediate effect.

Conclusion

Within the limitations of this in vitro study, intracanal medicaments significantly influenced the microhardness of root dentin. Calcium hydroxide paste produced the greatest reduction in dentin microhardness, followed by chlorhexidine gluconate gel. Aloe vera gel demonstrated the least reduction and preserved dentin microhardness most effectively among the tested medicaments. Therefore, Aloe vera gel may be considered a promising intracanal medicament for preserving the structural integrity of root dentin.

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Table 1: Intergroup comparison of mean Microhardness values among different groups

	N	Mean	Std. Deviation	F	P
Control	8	69.7875	0.60578	622.77	≤0.001*
Aloe vera gel	8	67.6875	0.33991		

Chlorhexidine gel	8	65.1000	1.20000		
Calcium hydroxide	8	53.8500	0.81766		

Graph 1: Comparison of mean Microhardness values among different groups

