

Comparative Analysis of Fracture Resistance of Tooth After Using Different Intracanal Medicaments: An in Vitro Study

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Abstract: Aim: To evaluate whether short-term (7 days) application of intracanal medicaments Calcium Hydroxide, Aloe Vera, Neem extract with Chlorhexidine, and N-acetylcysteine affects fracture resistance of human permanent teeth.

Materials and Methods: Forty, single-rooted human premolars of similar size were endodontically prepared using ProTaper rotary files up to F2. The teeth were divided into five groups (n=8): Group 1: Aloe Vera Gel, Group 2: Calcium Hydroxide, Group 3: N-acetylcysteine (NAC), Group 4: Neem + Chlorhexidine, Group 5: Control (no medicament). After sealing the apices, samples were stored at 37°C in saline for 7 days. Fracture resistance was tested and data were analysed via ANOVA and Tukey's HSD.

Results: Calcium Hydroxide had the highest fracture strength (1997.5 ± 174.9 N), statistically significantly higher than all other groups.

Conclusion: Calcium Hydroxide significantly enhances fracture resistance and is ideal when structural reinforcement is needed.

Keywords: Fracture resistance; Intracanal medicaments; Calcium hydroxide; Aloe vera; Neem extract; Chlorhexidine; N-acetylcysteine; Endodontics; Universal testing machine; Root canal therapy

1. INTRODUCTION

Intracanal medicaments play a crucial role in disinfecting root canals during multi-visit endodontic procedures. However, root fractures remain a major concern in endodontically treated teeth, often resulting from cumulative structural weakening due to factors like caries, endodontic access preparation, canal shaping, and extended use of medicaments.¹ Chemical agents and intracanal medicaments play a significant role in altering the fracture resistance of endodontically treated teeth.² Root fractures, when they occur, can drastically affect the long-term prognosis and success of treatment. Calcium hydroxide (CH) is a commonly employed intracanal medicament. Its therapeutic effect arises from the dissociation of calcium and hydroxyl ions, which occurs due to its solubility in substances like water, saline, Ringer's solution, and local anesthetics.³ These ions contribute to its antimicrobial and tissue-altering properties. Multiple studies have consistently demonstrated the efficacy of calcium hydroxide [Ca(OH)₂] in eliminating bacteria embedded within dentinal tubules.⁴ Calcium hydroxide remains one of the most extensively studied intracanal dressings due to its high pH and broad-spectrum antimicrobial action.⁵ Although it offers several therapeutic advantages, Calcium Hydroxide shows limited efficacy against persistent microbes like *Enterococcus faecalis* and *Candida albicans*. Additionally, its tendency to

degrade dentinal collagen may contribute to a progressive reduction in fracture resistance.

Yassen et al. found that even a short-term application of calcium hydroxide for one week can lead to a marked decline in dentin fracture resistance ranging from 19% to 30% attributed to the breakdown of collagen and proteoglycan structures within the dentin matrix.⁶

Aloe vera, in gel form, has emerged as a natural, biocompatible alternative with notable antimicrobial and anti-inflammatory properties.⁷ An in vitro investigation by Negin et.al into Aloe vera's intracanal use emphasized its lack of toxicity near root tissues, reinforcing its safety for periapical application.⁸

N-acetylcysteine (NAC), a thiol-based compound known for its antioxidant and mucolytic effects. A study by Quah et al. showed that NAC is bactericidal against *E. faecalis* when used at pH 11, effectively eradicating both planktonic bacteria and 21-day-old biofilms.⁹

Neem (*Azadirachta indica*), a traditional medicinal plant, offers a broad spectrum of therapeutic properties including antimicrobial, anti-inflammatory, and immune-enhancing effects, making it a valuable natural agent in endodontics.¹⁰

The study aimed to analyse whether short-term application of Calcium hydroxide, Aloe Vera, Combination of neem extract with chlorhexidine, N-acetyl cysteine in the root canal system for 7 days affects the fracture resistance of human permanent teeth.

Null Hypothesis proposed that there is no significant difference in the fracture resistance of human permanent teeth following the short-term (7 days) application of different intracanal medicaments Calcium Hydroxide, Aloe Vera, Neem with Chlorhexidine, and N-acetylcysteine compared to untreated controls.

2. MATERIALS AND METHODS

An In vitro study conducted using Forty mature, single-rooted human premolars in Universal testing machines.

The study included human permanent single-rooted premolars that were extracted for orthodontic purposes. Only teeth with fully formed apices and mature root development were selected. To ensure uniformity, teeth of similar root size and dentin thickness were chosen based on radiographic evaluation in both buccolingual and mesiodistal views. All selected specimens were free from caries, fractures, and restorations. Additionally, teeth with any history of previous endodontic treatment or internal resorption were excluded. A thorough visual and radiographic inspection was conducted to confirm the absence of crack lines or any structural defects, ensuring the integrity of each tooth prior to inclusion in the study. Convenience sample of 40 samples divided into five groups of six each.

3. ROOT CANAL PREPARATION

Access cavities were prepared in all specimens using a high-speed handpiece equipped with a No. 4 round bur. The working length was determined by inserting a file until its tip was visible just beyond the apical foramen, then subtracting 0.5 mm from that measurement. Initial canal negotiation was performed using a size 20 K-file up to the working length. Subsequent instrumentation was carried out with ProTaper NiTi rotary files (Dentsply Maillefer, Ballaigues, Switzerland) at a constant speed of 300 rpm, progressing sequentially from S1 to F2. During the preparation, 0.5 mL of 5.25% sodium hypochlorite was used to irrigate the canals between each instrument.

4. EXPERIMENTAL GROUP

Then the teeth were assigned to 5 groups randomly, with 8 teeth in each group.

- Group 1: Aloe Vera gel as intracanal medicament
- Group 2: calcium hydroxide as intracanal medicament
- Group 3: N-acetylcysteine as intracanal medicament
- Group 4: Neem extract with chlorhexidine vehicle as intracanal Medicament
- Group 5: No intracanal medicament

The apices of all the samples were sealed with acrylic resin, submerged in normal saline and put in a storage box at 37 degrees Celsius to mimic the oral environment. After 7 days, samples were removed from the storage box for fracture strength test.

Fracture strength Analysis

Each tooth was submerged in acrylic resin cylinder block to the cemento-enamel junction. The fracture strength was measured using a universal testing machine (Instron Universal Testing Machine). The

samples were fixed into a vice and the load was applied parallel to long axis of the tooth at 5 mm/min by cross-head speed. The maximum load required to fracture the samples was recorded and data were analysed statistically.

5. STATISTICAL ANALYSIS

The statistical analysis conducted in this study involved several key approaches to evaluate the fracture strength across different intracanal medicaments. Initially, an Analysis of Variance (ANOVA) was performed, yielding an F-value of 23.4426 and a p-value of 1.7278e-09, indicating a highly significant difference among the groups. To further investigate specific pairwise differences, a Tukey Honestly Significant Difference (HSD) post-hoc test was applied, providing adjusted p-values and confidence intervals to identify significant comparisons. Finally, a bar plot with error bars was generated to visually represent the mean fracture strength values and their standard deviations, facilitating a clear comparison across the groups of No Medicament, Aloe Vera, Calcium Hydroxide, N-Acetyl Cysteine, and Neem & Chlorhexidine. Statistical programming language python is used for the statistical analysis. A p value of <0.05 was considered statistically significant.

6. RESULT

Table 1 represent the mean fracture strength \pm standard deviation for each intracanal medicament group, calculated from eight measurements per group. Higher mean values indicate greater fracture strength, while larger standard deviations reflect greater variability within the group.

Table 1:

Sl. NO	Group	Mean \pm SD
1	Aloe Vera	1303.12 \pm 630.28
2	Calcium Hydroxide	1997.5 \pm 174.9
3	N-Acetyl Cysteine	650.75 \pm 91.82
4	Neem & Chlorhexidine	870.62 \pm 127.03
5	No Medicament	1026.38 \pm 96.41

*Mean and Standard Deviation Table of Summary of Fracture Strength by Intracanal Medicament.(original)

Table 2:

Group	Group	Mean Difference	P value	Lower limit	Upper limit
No Medicament	Calcium Hydroxide	971.125	<0.001*	534.2945	1407.9555
No Medicament	Aloe Vera	276.75	0.3779	-160.0805	713.5805
No Medicament	N-Acetyl Cysteine	-375.625	0.1204	-812.4555	61.2055
No Medicament	Neem & Chlorhexidine	-155.75	0.8421	-592.5805	281.0805
Calcium Hydroxide	Aloe Vera	-694.375	0.0005*	-1131.2055	-257.5445
Calcium Hydroxide	N-Acetyl Cysteine	-1346.75	<0.001*	-1783.5805	-909.9195
Calcium Hydroxide	Neem & Chlorhexidine	-1126.875	<0.001*	-1563.7055	-690.0445
Aloe Vera	N-Acetyl Cysteine	-652.375	0.0012*	-1089.2055	-215.5445

Aloe Vera	Neem Chlorhexidine	&	432.5	0.0534	-869.3305	4.3305
N-Acetyl Cysteine	Neem Chlorhexidine	&	219.875	0.6024	-216.9555	656.7055

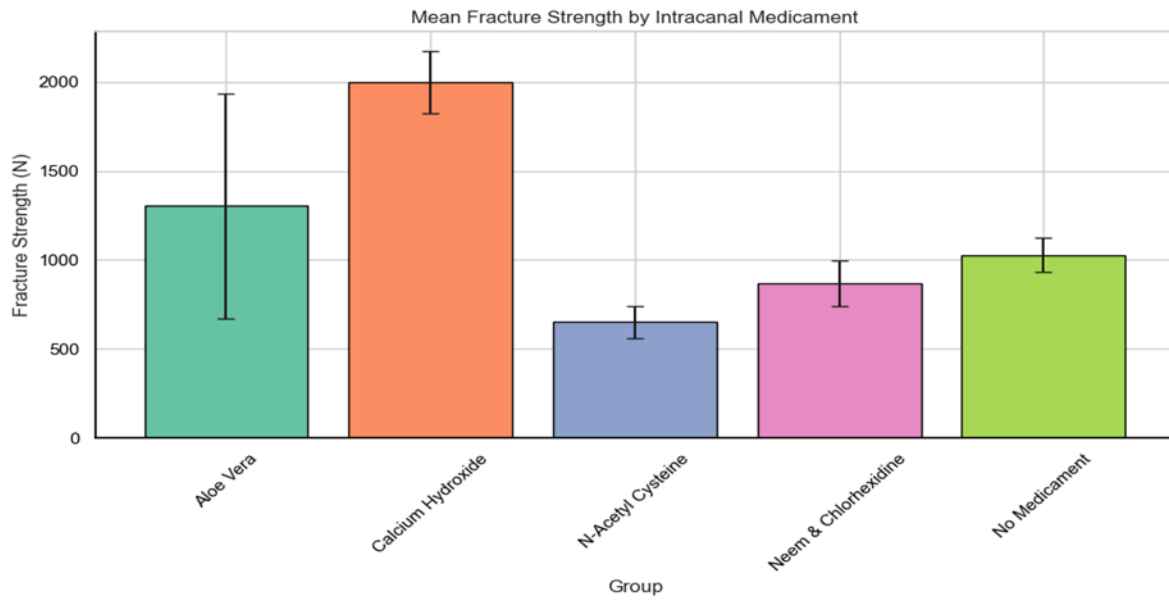
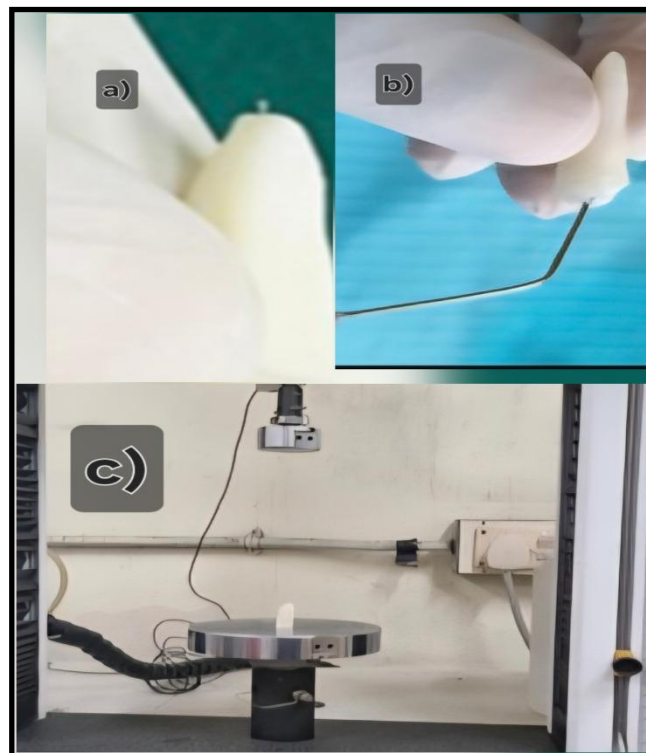


Figure 1: Mean Fracture Strength Comparison Across Intracanal Medicaments using bar charts and error bars(original)

Figure 2:a) Root canal Preparation b) Medicament placement c) Specimen in universal testing machine(



original)

0*Tukey HSD Table: Tukey HSD Pairwise Comparisons of Fracture Strength(original)

The ANOVA results ($F = 23.4426$, $p = 1.7278e-09$) demonstrate a highly significant difference in fracture strength across the groups, with a p-value well below 0.05. The Tukey HSD post-hoc test reveals that

Calcium Hydroxide significantly outperforms all other groups, showing the largest mean difference against N-Acetyl Cysteine (1346.75 N, $p = 0.0000$), followed by Neem & Chlorhexidine (1126.875 N, $p = 0.0000$), and Aloe Vera (694.375 N, $p = 0.0005$). Additionally, No Medicament significantly differs from Calcium Hydroxide (971.125 N, $p = 0.0000$), but not from Aloe Vera (276.750 N, $p = 0.3779$), N-Acetyl Cysteine (-375.625 N, $p = 0.1204$), or Neem & Chlorhexidine (-155.750 N, $p = 0.8421$).

No significant differences were observed between Aloe Vera and Neem & Chlorhexidine ($p = 0.0534$), N-Acetyl Cysteine and Neem & Chlorhexidine ($p = 0.6024$), or No Medicament and N-Acetyl Cysteine ($p = 0.1204$). However, Aloe Vera significantly differs from N-Acetyl Cysteine (652.375 N, $p = 0.0012$). These results indicate that Calcium Hydroxide consistently yields the highest fracture strength, while the other groups show more variable and less pronounced differences, with Aloe Vera and N-Acetyl Cysteine exhibiting notable distinctions in specific comparisons.

7. DISCUSSION

Calcium hydroxide being the commonly used intracanal medicament is ineffective against some of root canal micro-organisms particularly *E. faecalis*. Some of the herbal agents and recently introduced antimicrobial agents have added advantages of effectiveness against most of the root canal pathogens including *E. faecalis*. So, this study assessed and compared the fracture resistance of tooth after using these newer agents with that of Calcium hydroxide.

To ensure sample uniformity, premolar teeth with single roots and teeth of similar root size and dentin thickness were chosen based on radiographic evaluation in both buccolingual and mesiodistal views.

To mimic the oral environment, samples were stored in artificial saliva at 37 degree Celsius. To evaluate the fracture strength, the load was applied parallel to long axis of the tooth which could simulate normal occlusal forces.

Eun-Jung Shin et al showed that the calcium hydroxide placed in the root canal for 4 weeks weakened the strength of the root dentin approximately by 8.2% compared to the control group (no treatment).⁶ Sahebi et al. investigated how short-term exposure to calcium hydroxide affects the compressive strength of human permanent dentin. Using 50 extracted mature single-rooted mandibular premolars, they observed that dentin samples treated with calcium hydroxide for 30 days demonstrated reduced resistance to compressive forces.¹¹ The flexural strength of dentin is primarily dependent on the integrity of the bond between hydroxyapatite crystals and the collagen matrix, a connection maintained by acidic proteins and proteoglycans. Calcium hydroxide's alkalinity disrupts this bonding, potentially weakening the dentin.¹² This study revealed a significant difference in the mean fracture strength while using different intracanal medicaments. Kim D et al¹³ concluded that the antimicrobial effect of Ca (OH)₂ results from the release of hydroxyl ions when it comes into contact with aqueous fluids. Though calcium hydroxide (Ca(OH)₂) demonstrates broad-spectrum antimicrobial activity against many typical endodontic pathogens, it exhibits reduced effectiveness against *Enterococcus faecalis* and *Candida albicans*. Incorporating various vehicles or adjunctive agents can enhance the antimicrobial impact of calcium hydroxide. Similarly, Aloe vera showed remarkable antibacterial properties against *E. faecalis* biofilm.⁸ From the study by Mustafa M. et al¹⁰ concluded that the neem leaf extract has significant antimicrobial activity against *E. faecalis*, which shows comparable zones of inhibition with that of chlorhexidine and thus opens the perspectives for the use of neem extract as an intracanal medication. Chlorhexidine (CHX) is a commonly employed intracanal medicament in endodontics, valued for its potent antimicrobial properties and ability to suppress microbial proliferation within the root canal system.¹⁴ Recent studies have explored the use of N-acetyl cysteine (NAC) as an intracanal medicament, demonstrating its ability to inhibit the growth of *Enterococcus faecalis* and effectively disrupt its biofilm structure.¹⁵ N-acetyl cysteine (NAC) exhibits the strongest antimicrobial activity against *Enterococcus faecalis*, closely followed by *Aegle marmelos*, indicating that both agents hold promising potential as novel intracanal medicaments.¹⁶ It acts against all endodontic pathogens.¹⁷ It also provides immense protection of apical stem cells for regenerative endodontic procedures (REP's) for modern endodontics and gives an added advantage of being used as intracanal medicament.¹⁸

From various studies, it is clear that all the agents used in this study showed similar or superior efficacy as intracanal medicament. This study evaluated the fracture strength after using these agents on teeth. Calcium hydroxide group (group 2) showed superior fracture strength followed by aloe vera group (group 1) than the control group. While N-Acetyl Cysteine group (group 3) and neem extract & chlorhexidine group (group 4) showed deterioration in fracture strength of tooth.

However, there are limitations in this study that include being an invitro study, we cannot completely

recreate intraoral conditions, where occlusal forces are not continuous loading forces which is used in universal testing machine. Similarly, the shock absorbing effect of periodontal ligament to occlusal forces cannot be simulated here. The different agents used are not used in the same consistency and potency to stain the tooth is not addressed in this study. Although the vehicles used for delivering various intracanal agents are not specified in this context, previous studies have demonstrated that the vehicle used to deliver calcium hydroxide (CH) significantly influences the rate of ionic dissociation, thereby affecting how periapical tissues interact with the medicament, including the solubilization and resorption of the paste at varying rates.¹⁹

Only a short-term application of intracanal medicaments for 7 days was used in this study, so the results may vary on changing the duration. Intracanal medicament as calcium hydroxide and aloe vera increased the fracture resistance of tooth. Present study rejects the null hypothesis; fracture resistance of tooth varied with the use of different intracanal medicaments.

Furthermore, future studies are needed to comprehensively evaluate the effect of these agents on longer duration. The possibility of synergistic effect of calcium hydroxide and aloe vera with other agents can open future scope of intracanal medicaments.

8. CONCLUSION

Within the constraints of this study, Calcium Hydroxide significantly enhanced the fracture resistance of tooth and is ideal when structural reinforcement is needed followed by aloe vera.

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Nil.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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