Original Article

COMPARATIVE EVALUATION OF FRACTURE RESISTANCE OF ROOT DENTIN TO DIFFERENT INTRACANAL MEDICAMENTS: IN-VITRO STUDY

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ABSTRACT

Intracanal medication reduces micro-organisms after cleaning & shaping a root canal. Long-term exposure to some intra-canal medicament decreases fracture resistance of radicular dentin due to its strong alkalinity in long-term exposure. We studied the resistance of human root dentin to fracture following the application of intracanal medicament on the 7th and 10th days. Thirty-six human permanent extracted teeth with singleroot were collected. They were cleaned and shaped till Hand ProTaper F2. After de-coronation, roots were divided randomly as Group A - Calcium Hydroxide, Group B - Propolis, and Group C - Chitosan Mixed with Calcium Hydroxide, having 12 teeth respectively. The respective intracanal medicament paste was introduced in root canals. We divided samples into two subgroups. There were 6 teeth in each group. We checked the fracture resistance on the 7th and 10th days by using the universal testing machine. The fracture resistance was recorded in MPa automatically in the universal testing machine. We used one-way ANOVA followed by unpaired and paired for individual group comparisons. A p-value of Results- Mean values of fracture load (N) for 7days between groups $Ca(OH)_2$, Propolis, and Chitosan + $Ca(OH)_2$ was not significant Also, mean values of fracture load (N) at 10days when compared individually in group $Ca(OH)_2$, Propolis and Chitosan + $Ca(OH)_2$ there was no significant difference. Intracanal medicaments used decreased the resistance of radicular dentin to fracture. Propolis showed the least fracture resistance followed by Chitosan mixed with calcium hydroxide and Calcium Hydroxide alone.

Key words: Calcium hydroxide, Chitosan, Propolis, Fracture resistance, Human root dentin, In vitro study.

Introduction

Bacteria causes inflammation of the pulp and periapical region [1, 2]. Endodontic treatment aims to get rid of microorganisms from the canals [3, 4]. Prolonged endodontic success relies on debridement and disinfection of the canal. Persistent endodontic infection is due to the bacteria in the dentinal tubules, isthmi, ramifications, and the fin.

Biofilm formation ability of organisms hampers the complete disinfection of the canal [5]. Some microbes in these areas are not affected by chemo-mechanical preparation [6]. Use of intracanal medicament between the appointments helps in the complete elimination of these bacteria [7].

Calcium Hydroxide [$Ca(OH)_2$] has extensive use in dentistry owing to its broad antibacterial activity [8], as itliberates hydroxyl ions with a pH of 12.4 [9]. $Ca(OH)_2$ is most commonly used for short & long-duration medication (ICM). Researchers found that $Ca(OH)_2$ as an ICM can weaken the dentin compared to non-treated teeth [9]. However, activity is limited to a few days. This might be because the root canal milieu has organic and inorganic compounds [10]. In some studies $Ca(OH)_2$ when used as ICM for the long term reduces the organic component of dentin, influences the mechanical properties of dentin in the long-term ICM. It is stated that the strong alkaline pH of $Ca(OH)_2$ affects the dentin structure thereby causing poor fracture resistance.

Contemporary endodontics addresses the use of biological extract from plants and animals to reduce cytotoxic reactions. Propolis is a natural by-product of honeybee having flavonoids that have actions against bacteria, viruses, fungi, protozoa, against inflammation, and has antioxidant effects [11, 12]. Propolis contains flavonoids, esters, and aromatic acids which imparts antibacterial activity. Propolis is used in surgical wound healing in deep caries management, as root canal irrigant, in short term application is proven to be more efficient.

Chitosan an extract from alkaline deacetylation of chitin present in the shells of crustaceans is a (poly(1,4),-b-D-glucopyranosamine). It has antibacterial property biodegradable, biocompatible and non-toxic in nature [13]. Some studies have proven that chitosan when mixed with endodontic sealer and Ca(OH) 2 showed antibacterial activity against *E. faecalis* while chitosan-acetate solution

removed smear layer [13]. There are studies on Propolis and Chitosan showing antibacterial properties when used as an ICM. But there are very few studies in the literature that evaluated the resistance of human root dentin on fracture after application of ICM. Chitosan when mixed with Ca(OH)2 and sealer has proven antibacterial action against E. *faecalis*, used to remove smear layer [13].

So in this study, we have evaluated and compared the resistance of root dentin to fracture after using Ca(OH)2, Propolis, Chitosan, and Calcium Hydroxide mix as ICM.

Materials and Methods

We collected 36 freshly extracted permanent human single-rooted, non-carious, indicated for extraction. The crown portion was sectioned using a diamond disk, below the cementoenamel junction to obtain standard root length. The cleaning and shaping were done with Hand ProTaper till size F2, with in-between usage of 3% Sodium Hypochlorite, 17% EDTA, and normal saline as an irrigant. All the samples were finally irrigated with normal saline and dried using paper points. The roots were grouped into 3 having 12 teeth in each group to evaluate fracture resistance on the 7^{th}& 10^{th} days. They were further divided into two subgroups with 6 teeth.

Absorbent points were used to dry the canals. 12 root canals from each Group was received ICM, temporary cement was used to seal the access cavity and stored at 37ºC and in 100% humidity.

Group A: Calcium Hydroxide paste as an ICM (n=12). $Ca(OH)_2$ powder and saline drops were mixed.

Group B: Propolis paste as an ICM. (n=12). Propolis powder and saline drops were mixed.

Group C: Chitosan + $Ca(OH)_2$ as an ICM. (n=12). Chitosan solution was prepared by mixing 10% citric acid which was used as a vehicle for chitosan. This solution was mixed with $Ca(OH)_2$ powder.

All the ICM were mixed using a glass slab and spatula, as a creamy paste and introduced in the root canal with help of a sterile plastic syringe and needle having 20 gauge.

Bonded composite resin (3M ESPE) was used to seal root canals apically while cotton pellet and Cavit™ were used coronally (3M ESPE) to imitate the in vivo condition. All teeth were kept at 37ºC and 100% relative humidity. On the 7^{th} day, 6 teeth from each group were removed. All Roots were embedded in self-curing acrylic resin up to 5 mm of 2 cm diameter and 3 cm height of the block. Temporary Endo Restorative Material and cotton pellet were removed from the coronal part. The sample was mounted in a universal testing machine (Star Testing System) The punch with a tapered plunger of 1mm diameter and speed of 3 mm/min was applied at the center of the root until the specimen fractured. The load at which the specimen fractured was recorded automatically. On the 10^{th} day, the remaining 6 teeth from each group were removed and tested in the same manner as were checked for fracture resistance, on the 7^{th} day.

On the 7^{th} and 10^{th} days (n=6), roots were embedded in self-cure resin and mounted on the universal testing machine until the sample got fractured. The fracture resistance was recorded in MPa.

In the present study, descriptive statistics such as mean, SD, median, etc was done. Student's Unpaired t-test was used to compare the groups. The difference between 7 days and 10 days **(Table 1)** was done by Student's paired t-test. One way ANOVA test (Tukey-Kramer multiple comparison tests) was applied for all the groups compared together.

Results and Discussion

The mean of fracture load (± standard deviations) for the study groups **(Table 1)** on the 7^{th} day were - Group-A $Ca(OH)_2$ it was 170.12 ± 109.15 MPa, Group-B Propolis was 135.93 ± 40.10 MPa and Group-C Chitosan + $Ca(OH)_2$ was 184.40 ± 99.30MPa, respectively, without statistically significant differences in fracture load values between the groups ($p>0.05$). The student's unpaired "t" test showed no significant difference between mean values of fracture load (N) for 7days in groups $Ca(OH)_2$, Propolis, and Chitosan + $Ca(OH)_2$.

The result of the study revealed that the mean of fracture load (± standard deviations) for the study groups on the 10^{th} day - Group-A $Ca(OH)_2$ was 200.88 ± 43.02 MPa, Group-B Propolis it was 181.27 ± 50.43 MPa and Group-C Chitosan + $Ca(OH)_2$ was 221.10 ± 55.57 MPa, respectively, with no significant differences in fracture load values between the groups ($p>0.05$). After applying the Student's paired "t" test there was no significant difference between mean values of fracture load (N) for 10days in group $Ca(OH)_2$, Propolis and Chitosan + $Ca(OH)_2$.

Table 1. Fracture resistance (MPa) is measured as fracture load (N)

	Group A $Ca(OH)_2$ (n=6)	**Group B Propolis (n=6)**	**Group C Chitosan + $Ca(OH)_2$ (n=6)**

	Mean ± SD	Mean ± SD	Mean ± SD
On 7 days	170.12±109.15	135.93±40.10	184.40±99.30
On 10 days	200.88±43.02	181.27±50.43	221.10±55.57
Student's paired t-test value	2.1	1.8	2.0
'p' value and significance	P=0.093, not significant	p=0.106 not significant	p=0.097 not significant

After applying Student's paired t-test, there was no significant difference between mean values of fracture load (N) on 7 days and 10 days (Graph 1) in Group A: $Ca(OH)_2$, Group B: Propolis, and Group C: Chitosan + $Ca(OH)_2$.

Apical periodontitis is caused by bacteria, its elimination from the root canal is the basis for the healing of apical pathosis [14]. Disinfection and irrigation are essential to kill and remove microorganisms and smear layers from the canals and prevent reinfection [15, 16]. Some recently developed materials like Propolis and Chitosan are used as ICMs due to their antibacterial properties

Ca(OH)2 remains antibacterial till the high pH is maintained [17]. Sjogren *et al*. (1991) demonstrated that to get a negative culture Ca(OH)2 medicament should be applied for a period of 7 days [18]. Shuping *et al*. (2000) showed that almost 92.5% canal disinfection can be achieved by $Ca(OH)_2$ when kept at least for 1 week. $Ca(OH)_2$ powder alone is difficult to deliver, a liquid vehicle is required which helps to release hydroxyl ions. Aqueous solutions commonly used like sterile water or saline promote rapid ion liberation. The local anaesthetic solution being acidic can be used as a vehicle as $Ca(OH)_2$ is a strong base, minimally by acid [19].

The current endodontics shift is towards biological medication from plants and animals to reduce cytotoxic reactions. Folk medicine contains natural products -bee products like honey, pollen, propolis, fortified honey has been revitalized recently.

Propolis is composed of flavonoids, aromatic acids, and esters present in the resin.4% alcohol solution of propolis, when used as root canal filling, has shown a high success rate in acute and chronic eriodontitits [20]. Ethanolic extract of propolis acts against *E. faecalis*. and shown ICM as effective due to wide antibacterial action and low toxicity [21].

Chitosan is a compound of animal origin found widely in nature and has a fibrous structure. It is the basis of the outer skeleton of crustaceans like crabs, lobsters, and insects [22]. Chitosan has been used as an antimicrobial material against organisms -bacteria, algae, fungi, yeasts and in experiments involving *in vivo* and *in vitro* studies. Further investigation showed that chitosan is bacteriostatic rather than bactericidal [23]. Intracanal medicaments may hamper the physical characteristics and reduce their resistance to fracture. This study evaluated and compared the resistance of human root dentin to fracture using $Ca(OH)_2$, as well as Propolis and Chitosan, mixed with Calcium Hydroxide as ICMs [24, 25].

Root canal anatomy varies widely throughout the length of the canal with maximum variation. Thirty-six single-rooted and autoclaved extracted human teeth indicated for extraction were collected. Root length was standardized and canals were prepared till F_2 with 3% NaOCl as irrigant and final irrigation with 17% EDTA to remove smear layer followed by saline.

The result of the study revealed that the mean of fracture load (± standard deviations) for the Group-A $Ca(OH)_2$ (control group) at 7^{th} day, was 170.12 ± 109.15 MPa and at 10^{th} days it was 200.88 ± 43.02 MPa. This was chosen as the control group because $Ca(OH)_2$ is known to have a weakening effect on dentin, confirmed by the studies done by Rosenberg B and White JD. Sahebi S *et al*. and Andreasen JO. The strength of the dentine depends on the hydroxyapatite and collagenous fibrils link which can be disrupted by the strong alkaline PH of $Ca(OH)_2$ It collapses dentine structure as carboxylate and phosphate groups get denatured. The etiology of disruption could be because of neutralization, dissolution of proteoglycans and acid proteins which binds the collagen network and the hydroxyapatite crystals in dentin [26]. Andreasen evaluated the dentinal strength with a timeframe. It reached 50% within a year. The study result showed a reduction in the root strength after application of Ca(OH) 2 for 10 days (15% reduction in dentin strength) [27].

In this study, the mean fracture load (± standard deviations) for Group-B Propolis on the 7^{th} day was 135.93 ± 40.10 MPa and on the 10^{th} day, it was 181.27 ± 50.43 MPa. Though the lowest fracture resistance results were obtained with Group B, however statistically not significant on comparing to the control group ($p>0.05$).

Propolis is rich in flavonoids and phenolic acid, Phenolic acids being weak acids get adsorbed on hydroxyapatite molecules, resulting in surface complexation with hydroxyapatite [28]. in this s chemical reactions (equilibrium reactions) between a mineral surface and the solution, according to a study done by Jonsson C [29] which could be the cause for the significant reduction in fracture resistance for propolis.

In a study done by A.A. Elgendy and M.M. Nagy, the fracture resistance of root dentin was investigated and

compared by using the following ICMs, propolis, Triple antibiotic paste, and Chlorhexidine. They concluded that when Propolis is used as ICMs adversely affects fracture resistance of root canal dentin [29].

In this study, the mean of fracture load (± standard deviations) for Chitosan group-C at 7th day was 184.40 ± 99.30 MPa and at 10th day was 221.10 ± 55.57 MPa without significant differences in fracture load values as compared to control groups ($p>0.05$), which means fracture resistance of Group C is similar to control group.

Pimenta JA *et al*. [23] evaluated the microhardness of root dentin followed by 0.2% chitosan solution, 15% EDTA, and 10% citric acid. The experimental groups showed similarly reduced dentin microhardness ($p>0.05$) with a statistically significant difference on comparison with control (distilled water) ($p<0.01$). In the present study, all experimental groups showed reduced dentin microhardness. here 1% acetic acid was used for the preparation of the chitosan solution. However, in the current study, 10% citric acid was used. Citric acid is a known chelator that is used as a root canal irrigant for smear layer removal. However, it is known to reduce dentin microhardness.So, the decrease in microhardness of group C can be attributed to the vehicle used to dissolve chitosan, i.e 10% citric acid.

Conclusion

All the intracanal medicaments, that is, Ca(OH)2, Propolis and Chitosan mixed with calcium hydroxide, decreased the fracture resistance of radicular dentin. Amongst the three groups used, propolis showed the least fracture resistance followed by Chitosan mixed with calcium hydroxide and Calcium Hydroxide. However, the difference was statistically insignificant. Further long-term studies are required to check for their efficacy, and effect on radicular dentin.

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References

1. Leonardo MR, Silveira FF, Silva LA, TanomaruFilho M, Utrilla LS. Calcium Hydroxide Root Canal Dressing. Histopathological Evaluation of Periapical Repair at Different Time Periods. Braz Dent J. 2002;13:223-7.
2. Elsaka SE, Elnaghy AM. Antibacterial activity of calcium hydroxide combined with chitosan solutions and the outcomes on the bond strength of RealSeal sealer to radicular dentin. J Biomed Res. 2012;26(3):193-9.
3. Sundqvist G, Figdor D. Endodontic treatment of apical periodontitis. Oxford: Blackwell Science. 2003;2:242-87.
4. Lee Y, Han SH, Hong SH, Lee JK, Ji H, Kum KY. Antimicrobial efficacy of a polymeric chlorhexidine release device using in vitro model of Enterococcus faecalis dentinal tubule infection. J Endod. 2008;34(7):855-8.
5. de Paz LC. Redefining the persistent infection in root canals: possible role of biofilm communities. J Endod. 2007;33(6):652-62.
6. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res. 1981;89(4):321-8.
7. Byström A, Sunvqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J. 1985;18(1):35-40.
8. Sahebi S, Moazami F, Abbott P. The effects of short-term calcium hydroxide application on the strength of dentine. Dent Traumatol. 2010;26(1):43-6.
9. Moazami F, Sahebi S, Jamshidi D, Alavi A. The long-term effect of calcium hydroxide, calcium-enriched mixture cement and mineral trioxide aggregate on dentin strength. Iran Endod J. 2014;9(3):185-9.
10. Cuevas Guajardo S, Arzate Sosa G, Flores Chávez RI, Fabela González LV, MendietaZerón H. Antimicrobial activity with mixture of calcium hydroxide and propolis. Int J Pharma Bio Sci. 2011;2(4):203-10.
11. Ramani N, Mathew S. Comparative evaluation of antimicrobial efficacy of chlorhexidine digluconate and propolis when used as an intracanal medicament: ex vivo study. J Int Oral Health. 2012;4(2):17.
12. Jahromi MZ, Toubayani H, Rezaei M. Propolis: a new alternative for root canal disinfection. Iran Endod J. 2012;7(3):127-33.
13. Suzuki S, Masuda Y, Morisaki H, Yamada Y, Kuwata H, Miyazaki T. The study of chitosan-citrate solution as a root canal irrigant: A preliminary report. Oral Hyg Health. 2014;2:142.
14. Piovesani JF, Semenoff-Segundo A, Pedro F, Borges AH, Neves AN, MamedeNeto L, et al. Antibacterial capacity of different intracanal medications on Enterococcus faecalis. Dental Press Endod. 2012;2(2):53-8.
15. Darrag AM. Effectiveness of different final irrigation solutions on smear layer removal in intraradicular dentin. Tanta Dent J. 2014;11(2):93-9.
16. Shivanna V, Bhargavi R. Evaluation of the effect of calcium hydroxide and propolis intracanal medicaments on the push-out bond strength of a resin-based endodontic sealer to the root canal dentin: An in vitro study. Int J Oral Health Sci. 2015;5(2):87-92.
17. Saha S, Nair R, Asrani H. Comparative evaluation of propolis, metronidazole with chlorhexidine, calcium

hydroxide and curcuma longa extract as intracanal medicament against E. Faecalis–an invitro study. J Clin Diagn Res. 2015;9(11):ZC19.
18. Elgendy AA, Nagy MM. The effect of different intracanal medications on fracture resistance of root canal dentin. Tanta Dent J. 2015;12(3):163-7.
19. del Carpio-Perochena A, Bramante CM, Duarte MA, de Moura MR, Aouada FA, Kishen A. Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restor Dent Endod. 2015;40(3):195-201.
20. Ahangari Z, Eslami G, Ghannad S. In vitro antimicrobial activity of propolis in comparison with calcium hydroxide against Enterococcus Faecalis. J Dent Sch. 2012;30:9-17.
21. Hegde V, Kesaria DP. Comparative evaluation of antimicrobial activity of neem, propolis, turmeric, liquorice and sodium hypochlorite as root canal irrigants against E. faecalis and C. albicans–An in vitro study. Endodontology. 2013;25(2):38-45.
22. Doyon GE, Dumsha T, Von Fraunhofer JA. Fracture Resistance of Human Root Dentin Exposed to Intracanal Calcium Hydroxide. J Endod. 2015;31(12):895-7.
23. Pimenta JA, Zaparolli D, Pécora JD, Cruz-Filho AM. Chitosan: effect of a new chelating agent on the microhardness of root dentin. Braz Dent J. 2012;23(3):212-7.
24. Ajitha P, Rao CV, Lakshminarayanan L. Time dependent inhibitory effect of dentin on various calcium hydroxide medicaments-An in vitro study. Endodontology. 2003;15(2):334-6.
25. Estrela C, Estrela CR, Pécora JD. A study of the time necessary for calcium hydroxide to eliminate microorganisms in infected canals. J Appl Oral Sci. 2003;11(2):133-7.
26. Whitbeck ER, Quinn GD, Quinn JB. Effect of calcium hydroxide on the fracture resistance of dentin. J Res Natl Inst Stand Technol. 2011;116(4):743-9.
27. Milani AS, Rahimi S, Borna Z, Jafarabadi MA, Bahari M, Deljavan AS. Fracture resistance of immature teeth filled with mineral trioxide aggregate or calcium-enriched mixture cement: An ex vivo study. Dent Res J. 2012;9(3):299-304.
28. Üstün Y, Arslan S, Aslan T. Effects of calcium hydroxide and propolis intracanal medicaments on bond strength of resin-based endodontic sealer as assessed by push-out test. Dent Mater J. 2013;32(6):913-9.
29. Jahromi MZ, Tahmourespour A, Ziaei S. The effect of propolis on bacterial population isolated from necrotizing single canal tooth with chronic apical periodontitis versus chlorhexidine gluconate. J Med Plant Res. 2013;7(38):2873-8.