ORIGINAL RESEARCH

Effect of Intracanal Medicaments (Modified Triple Antibiotic Paste, Calcium Hydroxide, and Aloe Vera) on Microhardness of Root Dentine: An *In Vitro* Study

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Abstract

Aim: To compare the effect of three different intracanal medicaments, namely, modified triple antibiotic paste (MTAP), calcium hydroxide ($Ca(OH)_2$), and aloe vera, on the root dentine microhardness.

Materials and methods: A total of 50 extracted mandibular bicuspids were prepared using ProTaper Next rotary files. The roots of the bicuspids were alienated to three groups ($n = 10$ each) and one control group (untreated; $n = 20$). In three groups, the root canals were filled with MTAP, $Ca(OH)_2$, and aloe vera medicaments. After 21 days, medicaments were removed by Endo activator. Mean Knoop hardness numbers were calculated after treatment and compared with the untreated control group. Data were evaluated using the Student's *t* test (paired), ANOVA (one-way) followed, and the *post hoc* test.

Results: All treated groups except the aloe vera group had shown significant reduction ($p < 0.05$) in microhardness of the root dentin as compared with the untreated control group. The aloe vera group showed least reduction of microhardness and was statistically insignificant ($p > 0.05$).

Conclusion: Aloe vera shows promising results in terms of fewer effects on microhardness of the root dentin compared to MTAP and $Ca(OH)_2$.

Clinical significance: Elimination of most of the bacterial infection from the root canal and very minimum to no effect on the microhardness of the dentin in the root part are the basics of success in any endodontic treatment. Further *in vivo* studies are required to compare the efficacy of these intracanal medicaments.

Keywords: Aloe vera, Calcium hydroxide, Microhardness, Modified triple antibiotic paste, Root dentin.

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Introduction

Due to the polymicrobial environment of root canal infections, comprising both of aerobic and anaerobic microorganisms,[1] it becomes the need of the hour and utmost important to eliminate as many microbes as likely possible from root canals during root canal treatment. The intricate system of root canals makes it impossible toward decreasing the bacterial count just by cleaning plus shaping alone.[2] Endodontic instrumentation single-handedly cannot attain the sterile condition.[1] For further reduction of the microbial count, one of the important steps is usage of root canal medicaments.[3] A combination of instrumentation, irrigation, and intracanal medicaments would be considered ideal and is used in proceeding and doing root canal filling.[2,4]

A number of chemicals as intracanal medicaments have been tried. Out of these, the most preferred medicament regularly in the contemporary endodontic practice is calcium hydroxide ($Ca(OH)_2$) as others have been reported in the literature to cause biologic toxicity, allergic reactions, and resistance.[5] However, in resistant endodontic infections, the dominant bacteria is *Enterococcus faecalis*; this is even challenging to $Ca(OH)_2$. In such cases, the intracanal medicament of the choice is triple antibiotic paste (TAP).[6,7] The TAP is a combination of minocycline, ciprofloxacin, and metronidazole.[1]

It's been reported in the literature that continuous usage of $Ca(OH)_2$ and TAP drastically weakens the fracture resistance of the root. The microhardness of the tooth is determined by the composition of the dentin along with its surface structure.[3] The linkage between hydroxyapatite and collagenous fibrils controls and determines the dentinal strength.[8,9] A diminution in the strength of the treated tooth in terms of decrease in hardness of the treated tooth points toward collapse and dilapidation of tissues.[3,10] Over a considerable time period, it looks indispensable to build up endodontic materials, which could render the tooth structure stronger and less prone for fracture.[9]

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Owing to these disadvantages of TAP, recently substituting minocycline with clindamycin in the routine TAP has been shown to be successful in overcoming these disadvantages.[11] This modified triple antibiotic paste (MTAP) is nowadays being frequently used. It mainly contains clindamycin, metronidazole, and ciprofloxacin.[11]

In the exploration of a new antimicrobial compound, evidence of a better alternative has been shown among resources from traditional plants. The existence of an ample range of active phytochemicals, including terpenoids, lignans, flavonoids, polyphenolics, carotenoids sulfides, saponins, plant sterols, coumarins, phthalides, and curcumins, provides them highly anti-inflammatory, antioxidant, and antimicrobial properties. The additional presence of biocompatible features makes them appropriately useful to use in dentistry and endodontics specifically more extensively.[12] As compared to other natural extracts, aloe vera has extensive antibacterial commotion against a variety of oral pathogens,[13] which includes microorganisms in the dental pulp space which are resistant in nature, i.e., *E. faecalis* and *Candida albicans*.[14,15]

There are many disadvantages of the currently available intracanal medicaments. As maintaining the microhardness of the tooth and complete or near complete abolition of microbes existing within the root canals is a challenge, there is a need to identify the best intracanal medicament available.[2] Researches indicates that aloe vera is comparable to the regularly used medicaments like MTAP and $Ca(OH)_2$ in reducing the microbial count. However, studies testing consequences of aloe vera on microhardness of the root dentine are very scanty. Therefore this study was conducted to explore the outcome in terms of changes in the micro-hardness of the root dentin by utilizing three different intra-canal medicament namely (MTAP, $Ca(OH)_2$ and aloe vera).

Materials and Methods

The study was explained to the institutional ethic committee of the Banaras Hindu University before commencing this *in vitro* study. The study was done in Banaras, Uttar Pradesh, India. A total of 50 recently extracted one-rooted mandibular bicuspid teeth were chosen to perform the study. The inclusion criteria for sample selection involved teeth with straight and single canals, while teeth with caries, any fracture lines, curved canals, or calcified and obliterated canals were extruded.

It was assured that all the samples were calculus and debris free. Furthermore, before the start of the study, all the samples were disinfected using 2% chlorhexidine solution.

The crown structure of teeth was made to separate from the root the buccal part at the cementoenamel junction by a rotary bur (diamond), mounted on a high-speed hand piece. Exposing the dentin from its surface, which was subsequently turned flat and made perpendicular to the long axis of the tooth.

The instrumentation in the root canals was done with the help of ProTaper next rotary files (Dentsply Maillefer, Ballaigues, Switzerland) with F4 (size 40). Every time when the appliance was changed, irrigation of root canals was done with 2 mL 1% sodium hypochlorite (NaOCl) solution. The 5 mL 17% (EDTA) and 5 mL 5.25% NaOCl solution was used for final irrigation.

Overall four groups of the specimens were made based on the type of medicaments used, in which 20 teeth (control group) were not filled with any medicaments. Group I consisted of 10 samples in which MTAP was placed for around 14 days, 10 samples in group II for which $Ca(OH)_2$ was placed for 2 weeks, while in the group III of 10 samples aloe vera was placed for 14 days and group IV was the control group with 20 samples, of which 10 samples were tested immediately and rest 10 samples were tested after 14 days along with other groups.

The preparation of MTAP was done by mixing the powders of the various antibiotics and were compounded in equivalent parts of MTAP, which had metronidazole (Abbott India, India), ciprofloxacin (Arion Healthcare, Chandigarh, India), and cefaclor (Health Biotech, India), with the distilled water. To be used clinically, the antibiotic paste formulated was prepared with 1000 mg/mL solution to create slurry in the paste-like consistency, which could be exactly like the material used clinically.[16] $Ca(OH)_2$ (Sigma-Aldrich, Mumbai, India) was mixed in a ratio of 1.5:1 with sterile saline (weight/volume) so that a paste-like consistency could be obtained.[12] MTAP and $Ca(OH)_2$ were prepared in the paste form, while aloe vera was prepared in the gel form. The 100% raw aloe vera powder (Herbs and Crops Overseas, India) was used to prepare gel and for this methylcellulose was used as the vehicle.[17] Paste and gel were applied in the canal spaces and an attempt was made to make sure the medicament reached the apical foramen with the help of a lentulospiral. Temporarily, root canal access was sealed by a cotton pellet and Cavit (ESPE, Seefeld, Germany); following this, treated teeth were made to store at the temperature of 37°C and the humidity was maintained at 100% for 14 days. In order to simulate the usual clinical conditions following this, heated glue was utilized to close up the apices of the treated teeth.

After 14 days, the medicaments were removed from the root with the help of EndoActivator and NaOCl. Any leftover NaOCl was washed away with 2 mL of distilled water as a part of final irrigation for all the samples from all the groups. For drying the root canals paper points were used, followed by cutting the root canals in 2-mm sections transversally using a diamond disk; succeeding this process, resin blocks made up of resins were used to fix the roots. Baseline microhardness testing was concluded using a microhardness tester with a Knoop diamond indenter. All indentations were made according to American Society for Testing and Materials (ASTM) standard disc. Three indentations in series were made at certain points around the pulpal surface approximately 1 mm from the root canal. For every specimen, the mean Knoop hardness number (KHN) was calculated. All the steps starting from disinfection with chlorhexidine till acrylic resin block preparations were done by a single investigator whereas the testing of microhardness was carried by another investigator who was blinded about which intracanal medicament was used in which specimen.

After entering the data into the MS Excel (MS Office version 2007 developed by Microsoft, Redmond, WA), the analysis was done using the statistical software SPSS Version 20. Descriptive statistics were done by calculating mean and standard deviations (SD). The paired *t* test and one-way ANOVA were applied for testing the differences as applicable. The significance level was kept at below 0.05.

Results

Table 1 shows that the root dentin microhardness of the control group was 52.06 ± 10.88 which reduced to 48.39 ± 13.07 after 14 days, which was statistically not significant.

However, after 14 days, the root dentin microhardness of the MTAP group was reduced to 31.20 ± 9.58 and the $Ca(OH)_2$ group was reduced to 35.61 ± 9.01 when compared to the baseline and

Table 1: Comparison of root dentin microhardness of all the four groups with baseline by the paired *t* test

Group	*Time of evaluation*	*Mean*	*SD*	*p value*
Control	Before	52.06	10.88	0.571
	After 14 days	48.39	13.07	
MTAP	Before	52.06	10.89	0.003*
	After 14 days	31.20	9.58	
$Ca(OH)_2$	Before	52.06	10.89	0.010*
	After 14 days	35.61	9.01	
Aloe vera	Before	52.06	10.89	0.113
	After 14 days	45.14	3.71	

*p value < 0.05; significant

Table 2: Comparison of root dentin microhardness in all the four groups—control group, MTAP group, $Ca(OH)_2$ group and aloe vera group by one-way ANOVA

			95% confidence interval for mean			
	Mean	*Std. deviation*	*Lower bound*	*Upper bound*	*F*	*Sig.*
Control group	48.39	13.07	39.04	57.74	7.216	0.001*
MTAP	31.20	9.58	24.34	38.05		
$Ca(OH)_2$	35.61	9.01	29.16	42.05		
Aloe vera	45.14	3.71	42.48	47.80		
Post hoc test						
Group		*Group*			*p value*	
Control group		MTAP			0.001*	
		$Ca(OH)_2$			0.005*	
		Aloe vera			0.447	
MTAP		$Ca(OH)_2$			0.304	
		Aloe vera			0.002*	
$Ca(OH)_2$		Aloe vera			0.030*	

*p value < 0.05; significant

the results obtained were significant. While that of aloe vera group was reduced to 45.14 ± 3.71 after 14 days and the results obtained were statistically not significant.

Table 2 shows that the control group with maximum root dentin microhardness (48.39 ± 13.07), followed by the aloe vera group (45.14 ± 3.71), the $Ca(OH)_2$ group (35.61 ± 9.01), and least with the MTAP group (31.20 ± 9.5), and differences were found significant.

The *post hoc* analysis showed that statistically significant result ($p < 0.05$) was obtained when the control group was compared with MTAP and $Ca(OH)_2$. However, when the control group was compared with aloe vera, the results obtained were not significant ($p > 0.05$).

When the MTAP group was compared with the $Ca(OH)_2$ group, nonsignificant results were obtained. While, significant results were obtained when the MTAP group was compared with the aloe vera group ($p < 0.05$).

Similarly, when the $Ca(OH)_2$ group was compared with the aloe vera group, result obtained was significant ($p < 0.05$).

Discussion

The literature regarding root dentin microhardness of the endodontically treated teeth is in paucity in the Indian scenario. The present study can be considered as one of the few studies that explore the effect of certain intracanal materials on the root dentin microhardness.

In the present paper, the capability of the Knoop microhardness test was demonstrated toward detecting any surface changes within the dentin after treatment by MTAP, $Ca(OH)_2$, and aloe vera. After 14 days, least reduction of root dentin microhardness was found to be in the control group, followed by aloe vera, $Ca(OH)_2$, and MTAP groups.

In the present study, MTAP exhibited highest reduction in microhardness as compared to all different groups. The property may possibly be ascribed to the demineralizing property of antibiotic mixture, which is acidic in nature. Further explaining this feature, it would be likely decline in the phosphate/amide I fraction following MTAP treatment, which leads to demineralization when compared to untreated dentine.[11]

Although $Ca(OH)_2$ is most frequently utilized intracanal medicament, reduction in root dentin microhardness at the moderate to intermediate level has been reported by Yassen et al. in 2013.[10] Our findings were also in accord. The possible explanation for this destruction could be denaturing of the organic matrix or breakdown of the dentin inorganic structure due to the highly alkaline inorganic molecule with pH of 11.8.[10] Another reason for this reduction can be penetration into the intrafibrillar structure of mineralized collagen fibrils due to their minute molecular size, which cause changes in the three-dimensional confirmation of tropocollagen, leading to a diminished microhardness of the dentin.[18]

From the present study, it was observed that group that was treated with aloe vera demonstrated lowest reduction of root dentin microhardness. However, to prove the same, paucity of adequate data in literature regarding effects of aloe vera on microhardness of the root dentin still exists. Nonetheless, Sinha et al. in 2016[19] asserted that aloe vera had no unfavorable effect on shear bond strength of the dentin. In a randomized control trial done by Sinha et al. in 2016, aloe vera was used as a cavity disinfectant.[19] The results from the study conclude that aloe vera has antimicrobial efficacy against caries-causing microorganisms, i.e., *Streptococcus mutans*, in accordance to the study done by Bertolini.[20] But they have recommended to use aloe vera as a cavity disinfectant, whereas in the present study it was used as an intracanal medicament. Moreover, one of the recent studies has discovered that aloe vera also exhibits the matrix metalloproteinase (MMP) inhibitory effect against MMP 2 and 9.[21]

There are certain limitations of this study. First, it was an *in vitro* study. The study was performed on extracted teeth; the results may differ due to interaction of teeth with the oral environment and most importantly when the teeth are vital it may behave or may demonstrate various functions in different manners. And second, the result might vary if the medicament was filled till the root apex or not since the penetration depth of each medicament was not checked.

Conclusion

The elimination of most of the microorganisms within the root canal and very minimum to no effect on the microhardness of the dentin in the root part are the basics of success in any endodontic treatment. For reducing the population of microbes prior to filling in the root, the most crucial step is the use of root canal medicament. Various intracanal medicaments have been tried in endodontic treatment. Aloe vera shows promising results in terms of good antimicrobial properties and fewer effects on microhardness of the root dentin. More *in vivo* studies may be required to corroborate the same phenomenon.

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