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EFFECT OF 1% PHYTIC ACID, 5% PENTETIC ACID AND 6% MORINDACITRAFOLIA JUICE ON MICROHARDNESS OF THE ROOT CANAL DENTIN- AN IN VITRO COMPARATIVE EVALUATION

Endodontics

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ABSTRACT

Methodology:30 freshly extracted mandibular premolars with single canals were selected for the study. The samples were decoronated at the cemento-enamel junction. Roots were sectioned longitudinally into two halves. They were then polished and placed in autopolymerised resin moulds with the polished surface facing outside. The samples were divided into 3 groups based on the irrigants in which they were going to be immersed. Pre irrigation microhardness for all the samples were recorded at coronal,middle & apical third using Vickers hardness tester at 300 gm load and a dwell time of 20 seconds.Samples were immersed in 1% phytic acid , 5% pentetic acid and 6% morindacitrifolia juice for 15 minutes based on their group. All the specimens were again subjected to micro-hardness testing. Statistical analysis was done using one way analysis of variance (anova), post-hoc tukey test and independent student t-test to compare the pre and post immersion micro-hardness values of the selected samples. **Results:** The mean vickers hardness values in the coronal ,middle and apical 3rd region was the highest and statistically significant in morinda citrafolia group followed by phytic acid and least in pentetic acid. **Conclusion:** All tested irrigating solutions reduced microhardness of the radicular dentin at all levels. Reduction was least at the apical level for all the irrigants. 5% pentetic acid caused more reduction in dentin microhardness than 1% phytic acid while 6% Morinda citrafolia reduced the least.

KEYWORDS

1% Phytic Acid, 5% Pentetic Acid, 6% Morinda citrafolia, Irrigation, Micro hardness.

INTRODUCTION

Chemomechanical preparation combines the actions of endodontic instruments and irrigating solutions in order to remove pre-existing organic and inorganic debris left over from the mechanical preparation as well as to reduce the amount of microbial content and its by-products[1].In addition to the debriding action, irrigation serves the purpose of facilitating instrumentation by lubricating canals and by floating out dentinal filings[2].Various irrigating solutions have been used, such as: 0.5% -5.25% sodium hypochlorite, 3% hydrogen peroxide, 17% EDTA, and 2% chlorhexidine[2,3]. More recently 0.2% Chitosan and 6% Morindacitrifolia Juice (MCJ) are being considered for irrigation of root canal due to their antimicrobial properties and efficacy in removal of smear layer[4]. Herbal product (MCJ) is gaining in popularity for its natural remedies[5]. Murray PE et al., in 2008, evaluated 6% MCJ as an effectiveendodontic irrigants which performs was effective in removal of smear layer[6]. Its effectiveness in removing smear layer is better when compared to NaOCl but less than EDTA[4].

Another agent Phytic acid has a strong binding affinity to important minerals, such as calcium, iron, and zinc, although the binding of calcium with phytic acid is pH-dependent[7]. Nassar et al. observed that phytic acid was less cytotoxic and biocompatible than EDTA and that it could remove the smear layer from instrumented root canals and flat coronal dentin surfaces[8].

Pentetic acid also known as diethylenetriaminepentaacetic acid (DTPA) is a chelating agent whose chemical structure is an expanded version of EDTA. Like EDTA, pentetic acid is also an aminopolycarboxylic chelating agent, which forms stable and water-soluble complexes with divalent and trivalent metal ions[9]. In an interesting study by Qiu etal., it was found that pentetic acid has more antimicrobial activity against Gram-negative bacteria as compared to EDTA[10]. In a study by Gi etal., pentetic acid suppressed the elastase-mediated virulence of Pseudomonas aeruginosa more efficiently than EDTA[11].

The chemical structure of the dentin may change as a result of the irrigation solutions used during endodontic treatment, which may then affect the mechanical properties of the dentin. These irrigants that are used to remove the smear layer may have a similar effect on both the root dentin and the smear layer. Collagen is then exposed as a result, which ultimately results in a reduction in the micro-hardness of the dentin[4].Hence this study was designed to evaluate the effect of root canal irrigants solutions 1% phytic acid, 5% pentetic acid and 6% morindacitrafolia juice on microhardness of the root canal dentin.

MATERIALS AND METHODS

Sample Selection

Samples size was decided taking into consideration other similar in vitro studies. 30 freshly extracted non carious premolars were selected for the study and stored in 0.1% thymol solution. Non carious, non-fractured, non-restored, single rooted premolars were included in the study and the carious, fractured, restored and multi rooted teeth were excluded from the study.

Specimen Preparation

The selected samples were decoronated at the level of cemento-enamel junction with the help of a water-cooled diamond impregnated disc. No endodontic treatment was initiated for the samples. For longitudinal sectioning, grooves were created on the buccal and lingual external surface of roots without penetrating into the canals using a double-faced diamond disc under water cooling, and with the help of chisel, the roots were split into two halves. The sectioned specimens were then examined under a stereomicroscope to identify teeth with cracks. No teeth were found to have cracks; hence all the thirty samples were included in the study. All the samples were then ground polished with water cooled carborundum disc and finally polished with felt disc followed by buffing using 0.05 µm sized aluminium oxide powder mixed with distilled water. Freshly mixed auto polymerized resin was poured in plastic rings of uniform diameter. The specimens were embedded on the resin with the polished surface facing outwards. After curing of resin, the rings were removed and re-polishing of specimens was performed to remove excess material present on the tooth surface.

Grouping

All the samples were individually mounted on the stage of Vicker's micro-hardness tester and indentations were marked with a Vicker's diamond indenter at 300 gm load and dwell time of 20 seconds for measuring baseline data. Commercially available solutions of 1% phytic acid, 5% pentetic acid were used. 6% MCJ was freshly prepared by diluting 6 ml of MCJ { Cell Care} with 100 ml of normal saline using a pipette. All the three irrigating solutions were subjected to pH test, which was done using a digital pH meter and the pH readings were recorded.

30 samples were divided into groups of 10 samples each, and subjected to pre pretreatment Vickers hardness testing.

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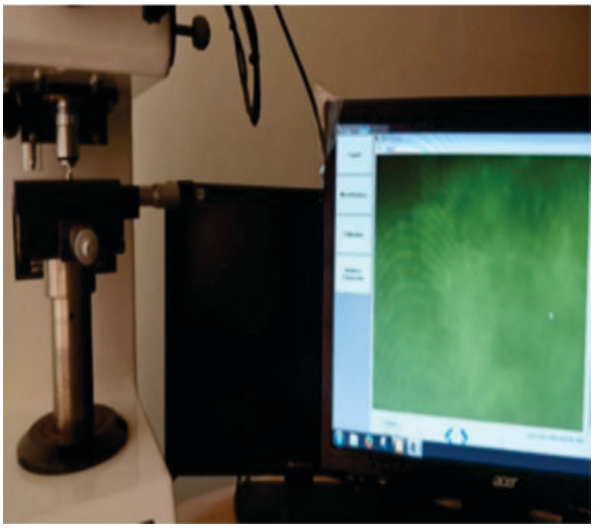
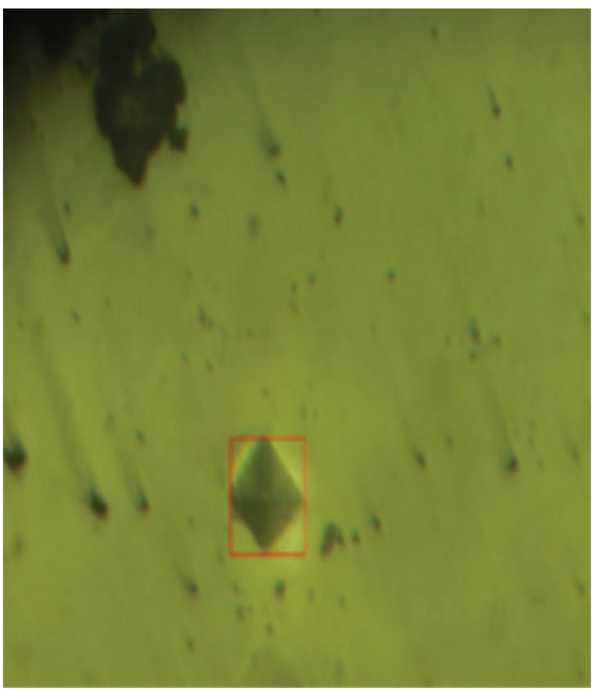
Group 1: 1.0% Phytic Acid
Group 2: 5.0% Pentetic acid
Group 3: 6.0% Morindacitrafolia juice

The specimens in these groups were then immersed in the respective irrigating solutions (1% phytic acid, 5% pentetic acid and 6% MCJ) for 15 minutes. At the end of the active treatment period of 15 minutes, the samples were rinsed with distilled water and dried. The samples were again mounted on the stage of Vicker's micro hardness tester and for each sample indentations were marked at three different locations with a Vicker's diamond indenter at 300 gm load and a dwell time of 20 seconds. All the indentations were marked in the middle region of the roots, approximately half way between the center of the canal lumen and the peripheral cementum where the dentin surface was uniform. These indentations were measured and converted into VHN values by the computerized monitor (Model- S1300 Micro Mach Technologies).Statistical analysis was carried out using one-way ANOVA (for intergroup comparison) along with Post-hoc Tukey test (to find out the pair-wise mean hardness difference between two groups) and independent student t-test (to compare the Mean micro-hardness and mean pH before and after immersion of the samples in their respective irrigants).



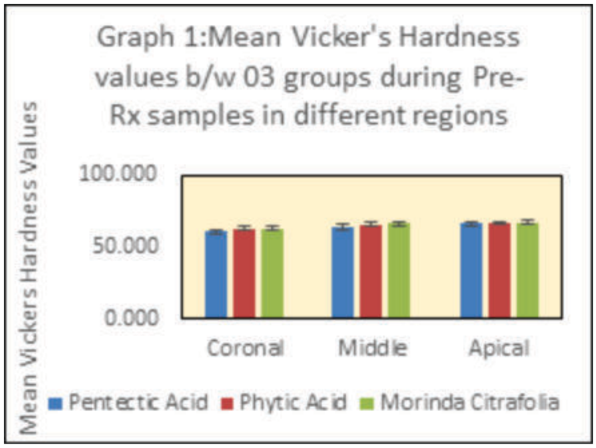


RESULTS

The mean Vicker's Hardness values for the pre-treatment period did not show significant differences between 3 groups at Coronal, Middle & Apical region. (Table 1).

Table 1: Comparison Of Mean Vicker's Hardness Values B/w 3 Groups During Pre-rx Samples In Different Regions Using One-way ANOVA Test

Comparison of mean Vicker's Hardness values b/w 03 groups during Pre-Rx samples in different regions using One-way ANOVA Test							
Region	Groups	N	Mean	SD	Min	Max	p-value
Coronal	Pentetic Acid	10	61.019	2.406	57.31	64.89	0.28
	Phytic Acid	10	62.912	3.804	58.64	69.61	
	Morinda Citrafolia	10	63.001	2.755	56.51	66.15	
Middle	Pentetic Acid	10	64.214	3.122	60.41	69.82	0.25
	Phytic Acid	10	65.851	3.797	62.35	73.22	
	Morinda Citrafolia	10	66.677	2.805	62.34	70.05	
Apical	Pentetic Acid	10	66.382	2.973	62.37	71.11	0.78
	Phytic Acid	10	67.054	2.805	61.13	71.17	
	Morinda Citrafolia	10	67.209	2.620	63.12	70.93	

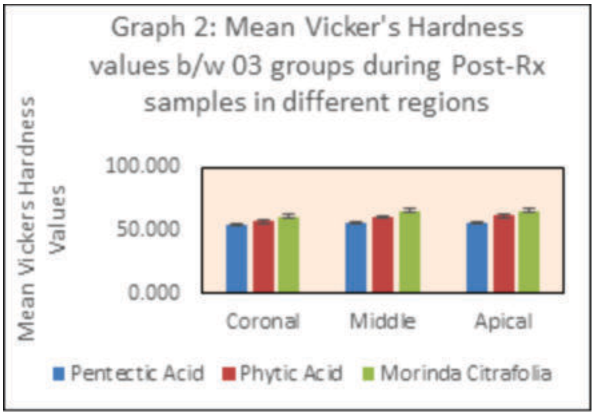


Pentetic acid group showed the mean value of 54.036 at coronal 3rd ,56.408 at middle 3rd ,56.640 at apical 3rd region. Phytic acid group showed mean value 57.077 at coronal 3rd,60.590 at middle 3rd ,61.759 at apical 3rd region. Morinda citrafolia group showed mean value of 61.039 at coronal 3rd,65.236 at middle 3rd ,65.757 at apical 3rd region. (Table 2).

Table 2:comparison Of Mean Vicker's Hardness Values B/w 03 Groups During Post-rx Samples In Different Regions Using One-way ANOVA Test

Comparison of mean Vicker's Hardness values b/w 03 groups during Post-Rx samples in different regions using One-way ANOVA Test							
Region	Groups	N	Mean	SD	Min	Max	p-value
Coronal	Pentetic Acid	10	54.036	1.842	51.22	56.68	<0.001*
	Phytic Acid	10	57.077	2.781	53.22	61.48	
	Morinda Citrafolia	10	61.039	2.557	55.1	64.41	
Middle	Pentetic Acid	10	56.408	1.567	54.67	58.91	<0.001*
	Phytic Acid	10	60.590	4.498	57.17	70.57	
	Morinda Citrafolia	10	65.236	3.106	59.97	69.15	
Apical	Pentetic Acid	10	56.640	2.871	53.21	61.97	<0.001*
	Phytic Acid	10	61.759	3.006	54.73	64.87	
	Morinda Citrafolia	10	65.757	2.361	62.63	68.64	

* - Statistically Significant



Multiple comparison between groups revealed that the Morinda Citrafolia group showed significantly highest mean Vickers hardness value as compared to Pentetic Acid & Phytic Acid groups(Table 3).

Table 3:multiple Comparison Of Mean Diff. In Vicker's Hardness Values B/w 03 Groups During Post-rx Samples In Different Regions Using Tukey's Post Hoc Test

Multiple comparison of mean diff. in Vicker's Hardness values b/w 03 groups during Post-Rx samples in different regions using Tukey's post hoc Test						
Region	(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff Lower	Upper	p-value
Coronal	Pentectic Acid	Phytic Acid	-3.041	-5.731	-0.351	0.02*
		Morinda Citrafolia	-7.003	-9.693	-4.313	<0.001*
	Phytic Acid	Morinda Citrafolia	-3.962	-6.652	-1.272	0.003*
Middle	Pentectic Acid	Phytic Acid	-4.182	-7.822	-0.542	0.02*
		Morinda Citrafolia	-8.828	-12.468	-5.188	<0.001*
	Phytic Acid	Morinda Citrafolia	-4.646	-8.286	-1.006	0.01*
Apical	Pentectic Acid	Phytic Acid	-5.119	-8.180	-2.059	0.001*
		Morinda Citrafolia	-9.117	-12.178	-6.057	<0.001*
	Phytic Acid	Morinda Citrafolia	-3.998	-7.059	-0.938	0.009*

* - Statistically Significant

The mean Vicker's hardness values in Morinda Citrafolia group during post treatment period showed relative differences in all 3 regions as compared to pre-treatment period and the mean differences in coronal, middle & apical 3rd regions were not statistically significant (Table 4).

Table 4: Comparison Of Mean Vicker's Hardness Values B/w Pre-rx & Post-rx Samples In Morinda Citrafolia Group In Different Regions Using Independent Student T Test

Comparison of mean Vicker's Hardness values b/w Pre-Rx & Post-Rx Samples in Morinda Citrafolia group in different regions using Independent Student t Test						
Region	Samples	N	Mean	SD	Mean Diff	p-value
Coronal	Pre Rx	10	63.001	2.755	1.962	0.12
	Post Rx	10	61.039	2.557		
Middle	Pre Rx	10	66.677	2.805	1.441	0.29
	Post Rx	10	65.236	3.106		
Apical	Pre Rx	10	67.209	2.620	1.452	0.21
	Post Rx	10	65.757	2.361		

The mean Vicker's hardness values in Pentetic Acid group during post treatment period showed significantly lesser values in all 3 regions as compared to pre-treatment period and the mean differences in coronal, middle & apical 3rd regions were statistically significant (Table 5)

Table 5:comparison Of Mean Vicker's Hardness Values B/w Pre-rx & Post-rx Samples In Pentetic Acid Group In Different Regions Using Independent Student T Test

Comparison of mean Vicker's Hardness values b/w Pre-Rx & Post-Rx Samples in Pentetic Acid group in different regions using Independent Student t Test						
Region	Samples	N	Mean	SD	Mean Diff	p-value
Coronal	Pre Rx	10	61.019	2.406	6.983	<0.001*
	Post Rx	10	54.036	1.842		
Middle	Pre Rx	10	64.214	3.122	7.806	<0.001*
	Post Rx	10	56.408	1.567		
Apical	Pre Rx	10	66.382	2.973	9.742	<0.001*
	Post Rx	10	56.640	2.871		

* - Statistically Significant

The mean Vicker's hardness values in Phytic Acid group during post treatment period showed significantly lesser values in all 3 regions as compared to pre-treatment period and the mean differences in coronal, middle & apical 3rd regions were statistically significant.(Table 6)

Table 6: Comparison Of Mean Vicker's Hardness Values B/w Pre-rx & Post-rx Samples In Phytic Acid Group In Different Regions Using Independent Student T Test

Comparison of mean Vicker's Hardness values b/w Pre-Rx & Post-Rx Samples in Phytic Acid group in different regions using Independent Student t Test						
Region	Samples	N	Mean	SD	Mean Diff	p-value
Coronal	Pre Rx	10	62.912	3.804	5.835	0.001*
	Post Rx	10	57.077	2.781		
Middle	Pre Rx	10	65.851	3.797	5.261	0.01*
	Post Rx	10	60.590	4.498		
Apical	Pre Rx	10	67.054	2.805	5.295	0.001*
	Post Rx	10	61.759	3.006		

* - Statistically Significant

This Infers that the mean vickers hardness values in the coronal ,middle and apical 3rd region was the highest and statistically significant in morinda citrafolia group followed by phytic acid and least in pentetic acid.

DISCUSSION

The quality of the instrumentation, irrigation, disinfection, and ultimately the obturation of the root canal system determines the long-term prognosis of root canal therapy. The effect of irrigating solution on the coronal and radicular root dentin during irrigation may cause changes to the physical and chemical characteristics of root canal dentin, including hardness[4].

Micro-hardness testing, one of the most straight forward and non-destructive techniques, is frequently used to explore small-scale changes in hardness. By using the Vicker's hardness test, the micro-hardness of dentin can be determined (for deep dentin). In order to assess the change in the surface of deeper hard tissue structures, Vicker's hardness test was employed because it is an appropriate and practical method. Because it provides incredibly exact readings and uses a single type of indentation for every type of surface treatment, this test is widely accepted[12].

The microhardness of the radicular dentin varied at different levels[13]and declined when the dentin was tested from the superficial to deep regions.Thus, in the present study, to measure the Vickers hardness values for the dentin, indentations were made in the coronal, middle, and apical thirds of the radicular dentin and were done at the 0.5-mm level from the root canal walls for standardization. For evaluation of a reduction in hardness, standardization was done by estimating the pre-treatment hardness of every sample and then comparing with the post-treatment hardness.

In the present study, the hardness of radicular dentin varied at the coronal, middle, and apical levels, which were well within the range documented in the literature andthe difference between the different levels was statistically insignificant ($P > 0.05$). The finding was in accordance with the previous study Patterson[13], Panighi et al[14] and Nikhil et al[15].

6% MCJ, which has been proven successful in removing smear layers has been tested in this study to evaluate its effect on the micro-hardness of dentin . The bioactive ingredients it contains are responsible for its antimicrobial properties. Additionally, it includes a few organic acids such caprylic acid, ursolic acid, and caproic acid[16][17]. The presence of these acids may also change the micro structure of dentin, which may be the cause of MCJ's little ability to remove smear layers[4]. This, in turn, may cause dentin's micro-hardness to decrease[18]. Root canal dentin's micro-hardness was not significantly reduced by 6% MCJ, but the acidic nature of the compound may have contributed to the observation of the slight drop in micro-hardness. The reduction in the microhardness was least compared to pentetic acid and Phytic acid. The study done by Saha et al, also demostarted the least effect on microhardness of dentin when MCJ was compared with 3%NaOCl,17%EDTA and 0.2% chitosan Phytic acid is highly negatively charged molecule that has affinity to Ca^{++}. Phytic acid was found to be more effective in removing the smear layer from NaOCl-treated flat coronal dentin surfaces and instrumented root canals than EDTA[19]. The pH of 1% phytic acid solution was around 1.2 in the study and this acidity, along with chelation ability, attributed to effective smear layer removal and Ca^{++} extraction. The Study done Nikhil et al, where 17% EDTA,0.2% chitosan and 1% Phytic acid were compared for their effect on microhardness of root dentin concluded that phytic acid reduced the least followed by 0.2% chitosan and 17% EDTA in microhardness. In this study 1%Phytic acid reduced the microhardness to a lesser extent than 5% Pentetic acid the action of which is similar to EDTA.

The data for microhardness of 5% pentetic acid is scarce; however, present study result showed least mean vicker's hardness value in coronal region with pentetic acid group. This study is first of its kind to present the microhardness of pentetic acid on dentinal wall. Pentetic acid being a chelating agent had equal efficacy as EDTA in terms of pushout bond strength[9]. Chelating agents are used during the biomechanical preparation of root canals to remove the smear layer, which increases the irrigants access to the dentin tubules and allows for adequate disinfection. Chelating agents also lessen the microhardness of the dentin, which facilitates the action of endodontic instruments. Previous study[9] showed comparable values of bond strength between 5% pentetic acid and 17% EDTA; this could be attributed to the fact toward pentetic acid having a similar structural composition as that of EDTA.

Only the microhardness of the dentin was assessed in this in vitro study; however, before using herbal irrigants regularly in vivo, more clinical research are necessary to determine their efficacy, safety, and biocompatibility.

CONCLUSION

All tested irrigating solutions reduced microhardness of the radicular dentin at all levels, Reduction was least at the apical level for all the irrigants. 5% pentetic acid caused more reduction in dentin microhardness than 1% phytic acid while 6% Morinda citrafolia reduced the least.

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