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A Comparative Analysis of the Impact of Different Intracanal Medicaments On Root Dentin Microhardness: An *In-Vitro* Study

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Abstract:-

➢ ***Aim***

To evaluate and compare the effect of 3 different intracanal medicaments namely calcium hydroxide (Neopex), Modified Triple Antibiotic Paste (MTAP) and Propolis on microhardness of root dentin.

➢ ***Materials and Methods***

Sixty (60), extracted mandibular premolar teeth were prepared using rotary files, decoronated and sectioned to obtain one twenty (120) mid-root dentin disc samples. These samples were randomly divided into three groups (n=40) and treated with Group I - calcium hydroxide $Ca(OH)_2$, Group II - Modified Triple Antibiotic Paste (MTAP) and Group III - Propolis. Twenty samples from each group were subjected to microhardness testing after 7 days of application of intracanal medicament and remaining 20 samples were subjected to microhardness testing after 14 days. Data obtained was statistically evaluated using ANOVA and Tukey's post hoc test.

➢ ***Result***

In the propolis group, microhardness values gradually increased ($p < 0.01$), but in the calcium hydroxide and MTAP groups, microhardness values gradually decreased ($p < 0.01$).

➢ ***Conclusion***

Compared to Calcium hydroxide and MTAP, propolis exhibits beneficial results in terms of having less of an impact on the microhardness of the root dentin.

➢ ***Clinical Significance***

The removal of bacterial infection from the root canal with little to no impact on the microhardness of the root dentin is essential for the success of endodontic treatment. Further in vivo studies are required to compare the efficacy of these intracanal medicaments.

Keywords:- *Calcium Hydroxide, Modified Triple Antibiotic Paste (MTAP), Propolis, Microhardness, Root Dentin, Intracanal Medicament.*

I. INTRODUCTION

Endodontic treatment's major goals are to eradicate the bacteria from the root canal system, neutralise their byproducts, and finally provide an environment that makes it impossible for any leftover microbes to flourish. [(1)] Biomechanical preparation and irrigation protocol in the RCT has shown to significantly decrease the count of bacteria in infected canals, however, it is difficult to accomplish total disinfection in many cases. [(2)] Intracanal medicament is an antimicrobial agent traditionally used in multi-visit endodontics to disinfect root canals between appointments especially in cases with severe periapical infection. [(3)]

➢ *Intracanal Medicaments Serve The Following Roles-*

- They eradicate any bacteria that might have persisted after canal instrumentation,
- They reduce periapical tissue inflammation and encourage tissue repair;
- In addition to neutralising tissue waste and rendering canal contents inert, it also aids in drying out persistently wet canals, often known as weeping canals. [(4)]

The type of medicament to be used is determined by the microorganism involved, their growth, and survival. [(5)] An intracanal medication should ideally be easy to administer and remove, have the desired antibacterial and anti-inflammatory properties, and offer a physical barrier against bacterial invasion. It needs to be biocompatible and non-toxic. The mechanical and physical characteristics of root dentine, including flexural strength, modulus of elasticity, and microhardness, should not be negatively impacted.[(6)]

➢ *Significance Of Microhardness- (6,7,8)*

- Microhardness is a measurement indicating mineral gain or loss in dental hard tissues and is measured by an indenter that penetrates microscopic areas.
- Mineral concentration and root dentin microhardness are frequently correlated.
- Root dentin demineralisation is reflected in decreased microhardness, and vice versa.

- Although a reduction in microhardness may make it easier to instrument the entire root canal, it will also reduce the dentin's flexural strength and modulus of elasticity, which could compromise and weaken the root structure.
- Additionally, a decrease in root dentine microhardness indicates softer root dentin, which impairs the sealing ability of obturating materials, leading to compromised prognosis of the endodontically treated tooth.

Since Hermann first introduced calcium hydroxide (CH) in 1920, it has been utilised extensively in endodontics as an intracanal medicament in between appointments. With a pH of about 12.5, it is a potent alkaline material with a number of biological characteristics, including antibacterial activity, tissue dissolving ability, suppression of tooth resorption, and induction of repair through hard tissue synthesis. The lethal effects of calcium hydroxide on bacterial cells are probably due to protein denaturation and damage to DNA and cytoplasmic membranes. However, the role of $Ca(OH)_2$ in eliminating microorganisms of resistant endodontic infections like E.faecalis and Candida species is uncertain. Furthermore, it has been observed that long term use of calcium hydroxide, negatively affects the fracture resistance and microhardness, thereby weakening the tooth. [9]

Triple antibiotic paste (TAP) was developed by Hoshino and colleague in the year 1996. Triple antibiotic paste is a combination of three medicaments, which are metronidazole (nitroimidazole compound), ciprofloxacin (synthetic fluoroquinolone), and minocycline (semisynthetic derivative of tetracycline). TAP intracanal medicament provides antimicrobial activity against most of the endodontic pathogens including E. Faecalis. Since studies have shown some shortcoming of this paste like development of bacterial resistance, allergic reaction, and crown staining due to the presence of minocycline. Another antibiotic, clindamycin has replaced minocycline in Modified Triple Antibiotic Paste (MTAP). Thus, MTAP a combination of metronidazole, ciprofloxacin, and clindamycin which is used as an intracanal medicament. [10]

Recently, natural alternatives of intracanal medicaments has gained popularity which includes Propolis, aloe vera, turmeric, babool, chitosan, green tea, etc.

Propolis (bee glue), is a flavonoid-rich natural by-product of honeybees. [6] It is a complex resinous mixture containing approximately 50% resin and balsam, 30% wax, 10% essential oils, 5% pollen and other chemical substances including amino acids, minerals, sugars, vitamins B, C & E, phenols and aromatic compounds. [11,12] The main component of propolis is phenolic compounds, which contain flavonoids; these compounds give propolis its biological properties. Propolis has been shown to possess antibacterial, antifungal, antiviral, anti-inflammatory, hepatoprotective, antioxidant, antitumor, and immunomodulatory effects. Several studies have recommended the use of Propolis for intracanal medications and irrigation solutions. Its other uses include- as an anticaries agent, a storage medium for avulsed tooth, a pulp capping agent, and a sealant for dentinal hypersensitivity. Propolis was proved to be effective against resistant endodontic pathogens. So, its use in endodontic treatment is of great importance especially against Enterococcus faecalis. [13,14,15] When applied to the external tooth surface, propolis has shown to increase the microhardness of the enamel. [16]

Few studies have examined the impact of propolis as an intracanal medicament on root dentin microhardness to date, and no statistical comparison of the effects of propolis, calcium hydroxide, and MTAP on root dentin microhardness has been assessed. Therefore, this study aimed to evaluate and compare the effect of 3 different intracanal medicaments calcium hydroxide (Neopex), Modified Triple Antibiotic Paste (MTAP), Propolis on microhardness of root dentin for 7 and 14 days.

II. MATERIALS AND METHOD

- Sixty (60), freshly extracted, mature, single-rooted mandibular premolar teeth of 18–40-year-old individuals were included in the study.
- An ultrasonic scaler was used to remove calculus, debris, and soft tissue remains from the teeth, and a 2% chlorhexidine solution was used to disinfect the teeth.
- All teeth were decoronated horizontally at the CEJ using a diamond disc to obtain sixty decoronated roots.
- Teeth were decoronated horizontally at the CEJ using a diamond disc to ensure a uniform root length of 14 mm (+\- 1mm).
- Root canal instrumentation of the decoronated roots was done with the help of rotary files until size 25-4%. Instrumentation was done under copious irrigation with 2mL of 3% sodium hypochlorite (NaOCl) and normal saline.
- To prevent the long-term effects of NaOCl and EDTA, the root canals were flushed with normal saline after receiving a final treatment of 5 mL of 5% NaOCl solution and 5 mL of 17% ethylene diamine tetra acetic acid (EDTA).

Fig 1: Root Length of 14 mm (+\- 1mm)

Fig 2: Teeth Decoronated Horizontally at CEJ

Fig 3: BMP of Decoronated Roots

➢ *Preparation of Root Dentin Disc Samples:*

- Were cut transversally into coronal third, middle third and apical third sections using a diamond disc under constant water cooling.
- After discarding the coronal and apical thirds of the roots, a 4 mm (+/- 1 mm) root section was chosen from the middle third of each root.
- These mid-root dentin cylinders were further sectioned longitudinally to obtain 2 dentin discs, thus making a total of 120 dentin disc samples from 60 teeth, these samples were mounted in autopolymerizing acrylic resin blocks leaving their dentin surface exposed.
- The dentin surface of the mounted specimens were ground flat and polished with 2500-grit abrasive paper.

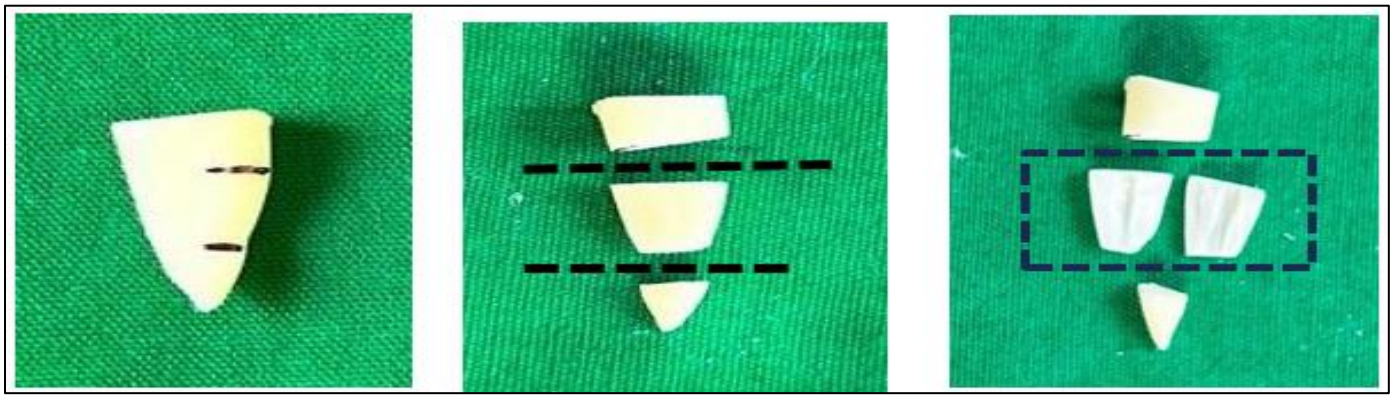

Fig 4: Root Cut Transversally into Coronal Third, Middle Third and Apical Third Sections

Fig 5: Mid-Root Dentin Disc Sample Mounted in Acrylic Resin

III. PREPARATION & APPLICATION OF INTRACANAL MEDICAMENT

All the 120 dentin disc samples were randomly divided into 3 experimental groups with 40-disc samples per group (n=40).

➢ *Group I: Calcium Hydroxide Paste (NEOPEX)-*
Commercially available $Ca(OH)_2$ with Iodoform paste was used. It was applied using lentulospiral.

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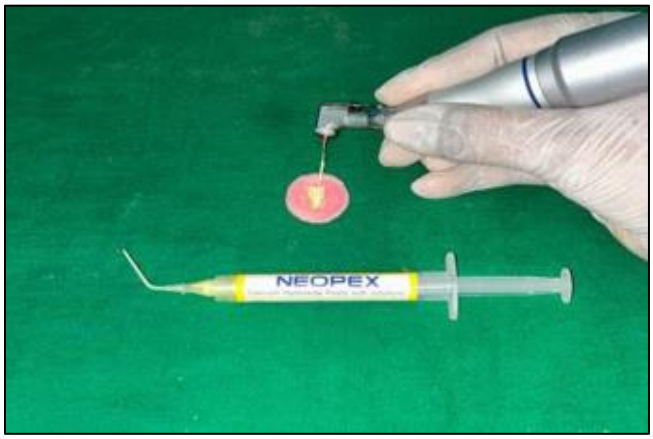

Fig 6: $Ca(OH)_2$ Medicament Application on Mid-Root Dentin Disc Sample

➢ *Group II: Modified Triple Antibiotic Paste (MTAP)*

MTAP was prepared by using 500 mg ciprofloxacin,400 mg metronidazole and 300 mg clindamycin after removing the enteric coating and crushing all the tablets separately in mortar and pestle. The crushed powder was passed through sieve to obtain fine powder particles. The powders obtained were weighed separately and mixed in a 1:1:1 proportion, with 1ml of saline to prepare a thick paste-like consistency of 1mg/ml concentration which was placed into the canal with the help of lentulospiral.

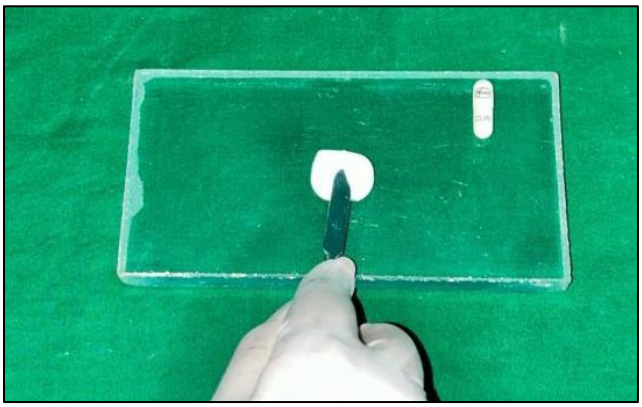
Fig 7: MTAP Preparation


Fig 8: MTAP Application on Mid Root Dentin Disc Sample

➢ *Group III: Propolis Paste-*

formed by mixing 1.5:1 (wt/vol) saline with commercially available propolis powder (Hi-tech Natural Products, India) to create a paste-like consistency, which is then introduced into the canal with the use of lentulospiral.

Fig 9: Propolis Paste Preparation


Fig 10: Propolis Paste Application on Mid Root Dentin Disc Sample

➢ *Laboratory Procedure*

- The dentin disc samples were kept in an incubator for up to seven and fourteen days in an airtight containers at 37^0 C and 100% humidity.
- Out of the 40 dentin disc samples in each group, 20 samples were subjected to microhardness testing after 7 days of application of intracanal medicament and remaining 20 samples were subjected to microhardness testing after 14 days of intracanal medicament application.
- Following treatment with medicaments, the samples were rinsed with distilled water and dried with absorbent paper before their microhardness was measured.

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➢ *Microhardness Assessment*

- A Vickers hardness indenter was used to create the indentations at a magnification of 40X. Three distinct indentations, each 100um deep, were formed in each root disc using a 200 g load and a 15 s dwell time, with a minimum of 500um between indentations.
- The indentations were positioned one millimetre away from the root canal wall.
- To determine the microhardness value (Vickers hardness number VHN), the length of the two diagonals was utilised.

Data obtained was recorded, tabulated and statistically analysed.

Fig 11: Vickers Micro hardness Testing Machine

Fig 12: Mounting of Specimen on Vickers Hardness Tester for Measurement

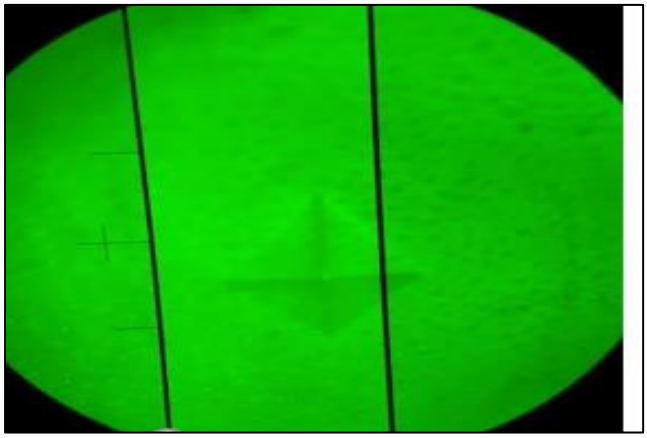

Fig 13: Photomicrograph of the Square Based Diamond Pyramid of the Indenter Impression on the Root Canal Surface

IV. RESULT

Table 1: Demonstrates the Descriptive Statistics for Microhardness of the Groups at Day 7 and 14.

Time	Group	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Day 7	Ca(OH)2	20	57.41	2.81	0.63	53.98	63.23
	MTAP	20	53.36	2.02	0.45	50.02	57.12
	Propolis	20	65.32	3.25	0.73	57.78	71.01
Day14	Ca(OH)2	20	45.05	2.81	0.63	40.11	50.11
	MTAP	20	34.86	2.81	0.63	30.34	40.22
	Propolis	20	67	2.22	0.5	62.34	70.11

Table 2: Comparison of Microhardness between the Groups at Day 7 and Day 14 using ANOVA TEST

		Sum of Squares	df	Mean Square	F	P- Value
Day 7	Between Groups	1480.22	2	740.11	98.29	0.000
	Within Groups	429.19	57	7.53		
	Total	1909.41	59			
Day 14	Between Groups	10793.14	2	5396.57	781.5	0.000
	Within Groups	393.61	57	6.91		
	Total	11186.75	59			

The P Value for both 7 and 14 days is 0.000 (< 0.05), hence the difference between the mean hardness of Ca(OH)2, MATP and Propolis is significant.

Propolis has highest value followed by Ca(OH)2 and MATP.

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Table 3: Multiple Comparison between the Groups at Day 7 and Day 14 Tukey Post Hoc Test

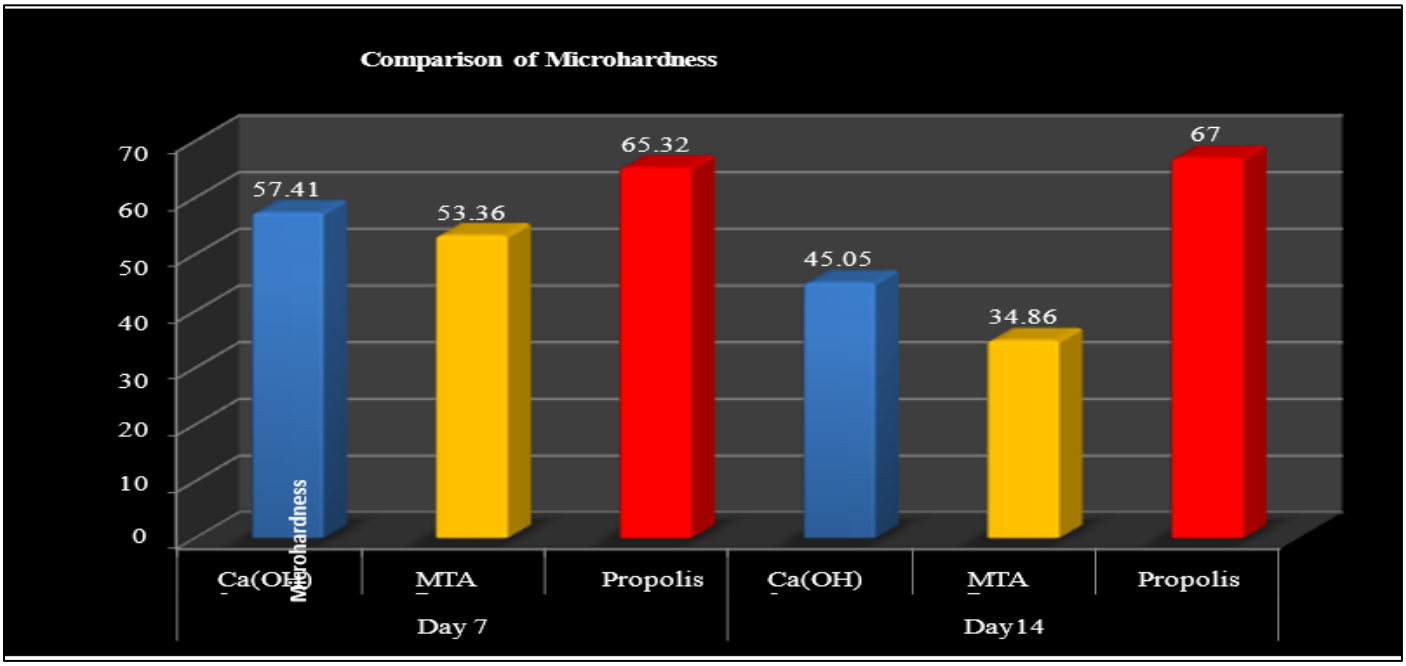
Multiple Comparison Day 7				
Group I	**Group II**	**Mean Difference (I - J)**	**Std. Error**	**Sig.**
Ca(OH)2	MTAP	4.05	0.87	0.000
	Propolis	-7.91	0.87	0.000
MTAP	Ca(OH)2	-4.05	0.87	0.000
	Propolis	-11.96	0.87	0.000
Propolis	Ca(OH)2	7.91	0.87	0.000
	MTAP	11.96	0.87	0.000

Table 4: Multiple Comparison between the Groups at Day 7 and Day 14 Tukey Post Hoc Test

Multiple Comparisons Day14				
Group I	**Group II**	**Mean Difference (I - J)**	**Std. Error**	**Sig.**
Ca(OH)2	MTAP	10.19	0.83	0.000
	Propolis	-21.96	0.83	0.000
MTAP	Ca(OH)2	-10.19	0.83	0.000
	Propolis	-32.14	0.83	0.000
Propolis	Ca(OH)2	21.96	0.83	0.000
	MTAP	32.14	0.83	0.000

The difference in mean hardness is significant in all the pairs at Day 7 and Day 14.

Following graph depicts the mean comparison of microhardness values among experimental groups at 2 different time intervals.



Graph 1: Comparison of Microhardness

V. DISCUSSION

In the Indian context, there is a dearth of literature on the root dentin microhardness of endodontically treated teeth, and research into the best intracanal medicament to improve root canal space disinfection and maintain the mechanical strength of the root dentin is still ongoing. The present study is one of the few that examines how a more recent intracanal medicament affects the microhardness of the root dentin.

The ability of the Vickers' microhardness test to identify surface alterations in the dentin following 7 and 14 days of treatment with Ca(OH)2, Propolis, and Modified Triple Antibiotic Paste (MTAP) was demonstrated in this study. Because Vickers microhardness testing is more sensitive to measurement errors, less sensitive to surface conditions, and capable of accurately testing small specimens like root discs, it was used in place of Knoop testing. (17)

Radicular tubular density and radicular dentin microhardness are inversely correlated, and tubular density of dentin has been reported to rise from cervical to apical portions of radicular dentin. (18) Therefore, in order to standardise each sample and prevent results from changing due to the variances in adjacent areas of the dentinal tissue, the microhardness test was carried out at the middle-third of the root structure, 1 mm from the root canal walls. Additionally, because older people's teeth have higher inter-tubular dentine microhardness, the age group for patients whose extracted teeth were included in this study was standardised between 18 and 40 years old in order to minimise this confounding factor, thereby excluding the chances of dentine or cementum deposition due to the aging process (19)

The current study's findings indicate that the propolis group experienced the least drop in root dentin microhardness, followed by the Ca(OH)2 and MTAP groups. Out of all the groups, MTAP showed the greatest decrease in microhardness. This could be due to the demineralizing property of antibiotic mixture, which is acidic (Ph= 2.9) in nature to preserve their chemical steadiness and to regulate their tonicity. Another explanation for this characteristic would be the probable decrease in the phosphate/amide I fraction after MTAP treatment, which causes demineralisation when compared to dentine that has not been treated. (20)

Despite being the most commonly used intracanal medication, Ca(OH)2 causes a mild to intermediate decrease in root dentin microhardness. Reason being, the denaturation of organic matrix of dentin due to the highly alkaline pH of Ca(OH)2 around 11.8. (21)

Because of their small molecular size, Ca(OH)2 can also penetrate into the intrafibrillar structure of mineralised collagen fibrils, causing changes in the three-dimensional confirmation of tropocollagen. This can lead to the breakdown of the protein structure and disruption of collagen fibre links, which in turn can negatively affect the mechanical properties of the root dentine, such as decreased microhardness and dentin's elastic modulus. (22) This could be a possible explanation for the reduced Vicker's Hardness number values in the calcium hydroxide group.

The findings showed that following propolis treatment, root dentine microhardness values increased steadily. This increases in dentine microhardness could be due to the ability of propolis to facilitate an intrafibrillar remineralization of demineralized dentine, enhancing its mineral content, and occluding the dentinal tubules. (23) Propolis's pH of 8.5 and bioflavonoids may be the cause, as they can form crystals inside dentinal tubules and aid in their obliteration. (24,25) Also, the re-mineralizing effect observed with propolis is not only attributed to the calcium phosphate precipitation derived from propolis, but is also due to the organic matrix stabilization provided by this product. (23) These might be reasons why the current study found that applying propolis to dentine enhanced its microhardness.

VI. LIMITATIONS

- It was an in-vitro study.
- Only the short-term effect (for up to 14 days) of application intracanal medicaments was assessed.
- Microhardness testing was limited to mid-root sections.
- Finally, based solely on microhardness testing, an estimate of the degree of dentine mineralisation cannot be made because the mineral content of dentine following the application of endodontic medicament was not assessed, which could account for the variations in microhardness values in all groups.
- Therefore, more precise studies like FTIR and assessment of the mineral content of dentine following propolis application need to be studied in order to have a better understanding of this phenomena.

VII. CONCLUSION

- The following conclusion can be made within the constraints of this study: when applied to root canals for up to 7 and 14 days, calcium hydroxide and modified triple antibiotic paste gradually reduce the root dentine microhardness.
- In contrast, the application of propolis to root dentine gradually increases its microhardness.
- Because of this, propolis was found to be a more effective intracanal medicament than calcium hydroxide and modified triple antibiotic paste without compromising the mechanical strength of the root dentine.

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