EJPMR, 2025, 12(11), 169-174 **Research Article** **Impact Factor 7.065**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

MICROHARDNESS OF HUMAN RADICULAR DENTIN AFTER CONDITIONING WITH DIFFERENT IRRIGANTS

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DOI: https://doi.org/10.5281/zenodo.17472218

How to cite this Article: S. Sunil Kumar, *Nagaswetha E., C. Sunil Kumar, Vamsee Krishna Nallagatla, K. S. Chandra Babu, R. Bharathi Suma. (2025). Microhardness Of Human Radicular Dentin After Conditioning With Different Irrigants. European Journal of Pharmaceutical and Medical Research, 12(11), 169–174.

Article Received on 01/10/2025 **Article Revised on 21/10/2025** **Article Published on 01/11/2025**

ABSTRACT

Introduction: Irrigating solutions used during root canal preparation to eliminate microorganisms can alter the chemical and physical properties of dentin, potentially increasing the risk of tooth fracture. Therefore, it is essential to select irrigants that offer maximum therapeutic benefits with minimal adverse effects. **Methodology:** Forty single-rooted maxillary central incisors were decoronated at the cementoenamel junction and sectioned longitudinally. Root segments were embedded in acrylic resin with the canal lumen exposed. Initial Vickers microhardness values were recorded, and samples were randomly assigned to four groups: Group 1 – Saline (control), Group 2 – 17% EDTA, Group 3 – Propolis, Group 4 – Bromelain. Each sample was treated with 2 ml of the respective irrigant for 5 minutes, rinsed with distilled water, and dried. Post-treatment microhardness was measured, and data were statistically analyzed. **Results:** All groups showed comparable baseline microhardness, highest in the propolis group (68.81 ± 4.36). Post-irrigation, all values decreased, with the greatest reduction in the propolis group (52.69 ± 4.69). Bromelain showed the least reduction, followed by EDTA and saline. ANOVA revealed significant differences in pre- and post-irrigation values ($P < 0.05$). **Conclusion:** All tested irrigants reduced root dentin microhardness in this in vitro study. Propolis caused the greatest reduction, while bromelain preserved microhardness closest to baseline. EDTA showed a moderate effect, similar to bromelain and saline. These results suggest bromelain may be a biocompatible alternative to traditional irrigants, preserving dentin integrity while potentially aiding smear layer removal.

KEYWORDS: Microhardness, EDTA, Dentin, Propolis, Bromelain, Vickers micro-hardness tester.

INTRODUCTION

Endodontic treatment aims to eradicate infection from the root canal system and to shape and disinfect the canal space without compromising the mechanical integrity of the dentin. While biomechanical instrumentation is crucial for debridement, it produces a smear layer that occludes dentinal tubules and may harbor residual bacteria. Hence, irrigation is essential not only for disinfection but also for smear layer removal and enhancing sealer adaptation.[1,2]

However, the chemical nature of irrigants directly influences the physico-mechanical properties of root dentin, including microhardness, which reflects the mineral content and structural integrity of the tissue. Dentin microhardness is an important parameter, as any reduction can adversely affect the tooth's resistance to fracture and impact the long-term success of root canal therapy.[3]

Ethylenediaminetetraacetic acid (EDTA), a commonly used chelating agent, is effective in smear layer removal due to its calcium-chelating property. However, EDTA has been shown to cause dentin demineralization, especially in the peritubular region, leading to a significant reduction in microhardness and potential weakening of the root structure.[4,5] Pashley emphasized that such demineralizing agents can disrupt the dentin collagen network, alter the mechanical properties of the substrate and compromise adhesion.[6]

To mitigate these adverse effects, natural alternatives with chelating and antimicrobial properties are being explored. Propolis, a resinous substance produced by bees, has demonstrated significant antibacterial, anti-inflammatory, and antioxidant properties. It contains flavonoids and phenolic compounds that may aid in smear layer removal without the extensive demineralization seen with EDTA.[7,8] In endodontics, propolis has shown promising results in preserving dentin microhardness and improving biocompatibility.[9]

Bromelain, a proteolytic enzyme extracted from *Ananas comosus* (pineapple), has emerged as a novel herbal irrigant. It exhibits anti-inflammatory and antimicrobial actions and can degrade organic components of the smear layer while being gentle on the mineral structure of dentin.[10] Initial investigations suggest that bromelain may reduce smear layer thickness and maintain dentin microhardness more effectively than conventional irrigants.[11]

Normal saline, though lacking antimicrobial or chelating properties, is often used as a control irrigant in endodontic studies. It is biologically inert and does not alter the dentin surface or microstructure, making it ideal for comparison in studies assessing the impact of active irrigants.[12]

Maintaining dentin microhardness during irrigation is crucial to prevent weakening of canal walls, which can lead to cracks, vertical root fractures, and reduced longevity of endodontic restorations.[13] Therefore, this study aims to evaluate and compare the effects of EDTA, propolis, bromelain, and normal saline on the microhardness of root dentin, to identify irrigation protocols that ensure effective cleaning while preserving dentin integrity.

MATERIALS AND METHOD

Sample preparation

40 single-rooted teeth were extracted which are free from restorations, cavities, or fracture. Teeth were stored in a 5.2% sodium hypochlorite solution to remove/ eliminate the hard deposits. Each tooth is decoronated at the cementoenamel junction. [Figure 1] A diamond disc was used to create grooves along the long axis of the roots. [Figure2] the roots were then longitudinally split in a buccolingual direction with a chisel to expose the root dentin surface. The root segments were embedded in auto polymerizing acrylic resin with a canal lumen facing out. [Figure3] to remove surface irregularities, a series of emery papers were used. Before irrigation initial microhardness values were measured for each sample using the Vickers microhardness test. Using a 50-g (HV 0.05) load and a 10-second dwell duration, indentations were made parallel to the margin of the root canal lumen, i.e., 0.5mm away from the root canal. [Figure 4] To hold the irrigant, construct a wax basin-like model on the exposed root dentin surface.

Preparation of the solutions

Preparation of 4% propolis solution using 500 mg propolis and distilled water

One 500 mg tablet of propolis dissolved in 120 ml of warm distilled water=4%propolis solution

Preparation of 4% Bromelain solution using 500 mg Bromelain and distilled water

One 500 mg tablet of Bromelain dissolved in 120 ml of warm distilled water=4% Bromelain solution.

The specimens were randomly divided into 4 groups according to the irrigants used.

Group 1 (n=10): The specimens were treated for 5 minutes with 2 ml of saline solution (Control group)

Group 2 (n=10): The specimens were treated for 5 minutes with 2 ml of 17% EDTA solution.

Group 3(n=10): The specimens were treated for 5 minutes with 2 ml of 4% Propolis.

Group 4(n=10): The specimens were treated for 5 minutes with 2 ml of 4% Bromelain.

After the samples were treated with irrigants, post application value of Vickers microhardness (Vickers hardness number [VHN]) was measured at the surface of the dentin of each sample using the same parameters as done for the baseline values (Fig. 4).

After surface treatment, the specimens were rinsed with distilled water and blotted dry. After irrigation, post-microhardness values were recorded for each sample adjacent to the initial.

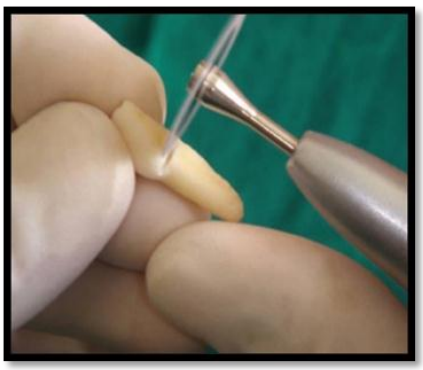
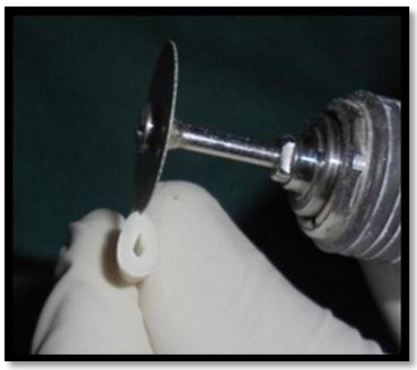


Figure (1)



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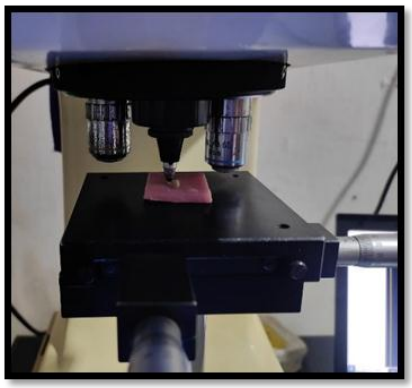

Figure (3)

Figure (4)

Statistical analysis

Pre-microhardness (Table 1) and post-microhardness (Table 2) values were tabulated and statistically analysed by one-way ANOVA and the intergroup comparison of mean microhardness was conducted using a post hoc Tukey's test. Significance was established at $P< 0.03$ value.

RESULTS

Pre-Irrigation Microhardness Values

The mean Vickers microhardness values of root dentin before irrigation for each group are summarized in Table 1. All groups exhibited comparable baseline microhardness values. The highest mean microhardness was observed in the propolis group (68.81 ± 4.36), followed by EDTA (66.58 ± 3.89), bromelain (65.18 ± 4.13), and saline (61.50 ± 2.27).

Statistical analysis using one-way ANOVA revealed a significant difference in pre-irrigation microhardness among the groups ($F = 3.533$, $P = 0.026$). However, the difference was not substantial enough to affect post-treatment comparisons due to narrow standard deviations.

Post-Irrigation Microhardness Values

After irrigation, all groups demonstrated a reduction in dentin microhardness, as shown in Table 2. The propolis group exhibited the greatest decrease, with a mean post-irrigation value of 52.69 ± 4.69, followed by saline (57.20 ± 1.80), EDTA (57.71 ± 4.40), and bromelain (57.72 ± 3.71).

One-way ANOVA again indicated a statistically significant difference in post-irrigation microhardness among the groups ($F = 3.423$, $P = 0.030$). Despite being a chelating agent, EDTA caused less reduction in microhardness than propolis, while Bromelain maintained microhardness values most closely to the pre-treatment level.

Table 1: Mean and Standard deviation value of microhardness for each group before irrigation.

		N	Mean	Std. Deviation	F value	*P* value
PRE	SALINE	10	61.500	2.273	3.533	0.026*
	17% EDTA	10	66.580	3.885		
	PROPOLIS	10	68.810	4.363		
	BROMELAIN	10	65.180	4.133		

Table 2: Mean and Standard deviation value of microhardness for each group after irrigation.

		N	Mean	Std. Deviation	F value	*P* value
POST	SALINE	10	57.200	1.798	3.423	0.030*
	17% EDTA	10	57.710	4.399		
	PROPOLIS	10	52.690	4.689		
	BROMELAIN	10	57.720	3.709		

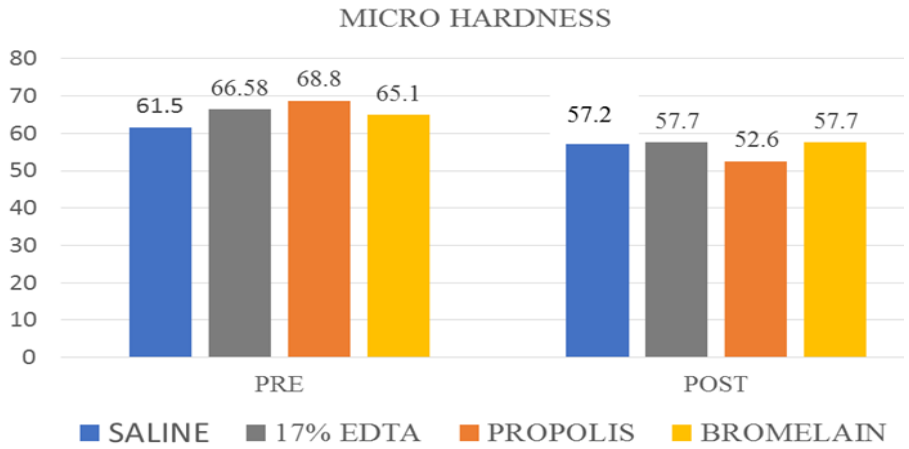

Figure (1): Bar diagram showing mean microhardness for different irrigant.

Table 3: Intergroup comparison of mean microhardness values was analyzed using Post-hoc Tukey's test.

Dependent Variable	GROUPS	GROUPS	Mean Difference	Significance
POST	SALINE	17% EDTA	-.51000	0.997
		PROPOLIS	4.51000	0.268
		BROMELAIN	-.52000	0.996
	17% EDTA	SALINE	.51000	0.997
		PROPOLIS	5.02000*	0.048*
		BROMELAIN	-.01000	1.000
	PROPOLIS	SALINE	-4.51000	0.268
		17% EDTA	-5.02000*	0.048*
		BROMELAIN	-5.03000*	0.048*
	BROMELAIN	SALINE	.52000	0.996
		17% EDTA	.01000	1.000
		PROPOLIS	5.03000*	0.048*

Interpretations: The mean microhardness of dentin 17% EDTA differs significantly with Propolis, the Mean microhardness of Propolis differs significantly with 17% EDTA and Bromelain, the mean micro-dentin hardness of Bromelain differs significantly with Propolis

DISCUSSION

Dentin microhardness is a critical parameter that reflects the structural and mechanical integrity of dentin, which is essential for the long-term success of endodontically treated teeth. Irrigation plays a pivotal role in endodontic treatment by disinfecting the canal, removing the smear layer, and dissolving necrotic tissue. However, the interaction of irrigants with dentin, particularly the superficial canal walls, can significantly alter the mechanical properties of the dentin substrate.[6,14]

Cruz-Filho et al.[15] emphasized the importance of simulating clinical conditions in laboratory studies. In the present study, longitudinally sectioned specimens were used to maximize the surface area of dentin exposed to the irrigants, thereby replicating the real-time interaction between root canal dentin and irrigation solutions.

The 5-minute contact time chosen in this study was based on findings by Ulusoy and Görgül[16] and Sayin et al.[17] who suggested that this duration is sufficient for smear layer removal and antimicrobial activity without excessively compromising dentin structure. Our results confirmed that all tested irrigants-EDTA, propolis, bromelain, and saline-led to a reduction in dentin microhardness, although to varying degrees.

Among the tested irrigants, 17% EDTA caused a significant decrease in dentin microhardness. This finding aligns with studies by Ari et al., De-Deus et al., Eldeniz et al., Sayin et al., and Thangaraj et al., who

demonstrated that the chelating action of EDTA causes demineralization and weakening of the root canal dentin, especially with increasing exposure time.[4,5,18,19,20] Calt and Serper[21] reported that prolonged exposure (more than 10 minutes) to EDTA leads to erosion of both peritubular and intertubular dentin and recommended limiting its application to less than one minute to prevent such damage. The mechanism involves the removal of calcium ions and the organic matrix from intertubular dentin, leading to increased tubular diameter and decreased hardness.[22]

In the current study, 4% propolis also significantly reduced microhardness. Elgendy[23] reported similar findings, attributing this to the phenolic components in propolis, which can chelate calcium and interact with hydroxyapatite, disturbing the mineral equilibrium. Although Bhagwat et al.[24] found that propolis caused less reduction in microhardness than EDTA and CHX, the current results demonstrated a greater reduction. This discrepancy may be due to differences in methodology, sample preparation, or the concentration and formulation of the propolis extract.

Contrary to expectations, propolis, despite its natural origin and recognized antimicrobial and anti-inflammatory properties, showed the highest reduction in dentin microhardness. While Parolia et al. (2010) reported that propolis has favorable biological effects, including antibacterial and antioxidant activity.[7] its acidic nature and the presence of polyphenols may have contributed to demineralization of the dentin surface. Hegde et al. (2020) found that propolis can interfere with dentinal substrate through tubule occlusion, which may influence surface properties like hardness.[9]

Bromelain, a proteolytic enzyme derived from pineapple (*Ananas comosus*), showed the least reduction in dentin microhardness among the experimental groups. This enzyme is rich in cysteine residues, which may act as mild chelating and reducing agents, potentially protecting the dentin matrix from aggressive demineralization.[11] Bromelain's broad specificity for protein cleavage and its biocompatibility make it a promising alternative to conventional irrigants, as it appears to effectively remove organic tissue while preserving the mechanical strength of dentin.

The saline group, used as a control, it lacks antimicrobial or chelating properties and demonstrated a slight reduction in dentin microhardness, likely due to prolonged moisture exposure rather than any chemical action. Similar results were reported by Slutzky-Goldberg et al. (2004), who noted minor softening in dentin specimens stored in aqueous media over time.[12]

These findings emphasize the importance of selecting irrigants that not only disinfect effectively but also preserve the mechanical integrity of root dentin. Excessive reduction in microhardness can lead to increased susceptibility to fracture, reduced bonding of obturating materials, and overall compromised prognosis of root canal therapy. Saleh et al. (2002) highlighted that pretreatment-induced dentin changes significantly influence sealer adhesion, further reinforcing the need for biocompatible irrigation protocols.[13]

In summary, while EDTA remains a gold standard for smear layer removal, its significant impact on dentin hardness warrants caution in its clinical application. Herbal alternatives like bromelain and propolis may offer safer alternatives, but further research is required to optimize their concentration and clinical efficacy.

Limitations

- The study was conducted in vitro; clinical conditions such as intracanal pressure, temperature, and complex anatomy may influence outcomes.
- Long-term effects on the bonding of sealers or fracture resistance were not assessed.
- Only one concentration per irrigant was tested; future studies should explore dose-dependent effects.

CONCLUSION

Within the limitations of this in vitro study, all tested irrigants caused a reduction in root dentin microhardness. Propolis resulted in the most significant decrease, while bromelain preserved microhardness values most closely to baseline. EDTA, although an established chelating agent, showed a moderate effect comparable to bromelain and saline.

These findings suggest that bromelain may serve as a promising biocompatible alternative to traditional irrigants, effectively preserving dentin integrity while potentially aiding in smear layer removal. Further clinical studies are required to validate its long-term effects and antimicrobial efficacy in vivo.

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