ORIGINAL ARTICLE

Turk Endod J 2025;10(3):218–223
doi: 10.14744/TEJ.2025.48379

Effect of dual rinse® HEDP, propolis and EDTA on root dentin microhardness: An in vitro study

Birgül Özaşır, Tufan Özaşır, İzel Yeşil, Zeynep Serra Arslan, Kamran Gülşahı

Department of Endodontics, Baskent University Faculty of Dentistry, Ankara, Türkiye

Purpose: The aim of this in vitro study was to compare the effects of root canal irrigation agents—Dual Rinse HEDP, Propolis, and EDTA—on root dentin microhardness.

Methods: Forty human maxillary central incisors were sectioned buccolingually to yield 80 specimens. The samples were embedded in acrylic blocks; their surfaces were sanded and polished. Thereafter they were randomly assigned to four groups (n=20 each): Saline, EDTA, DR HEDP, and Propolis. Vickers microhardness measurements were performed before and after irrigation using a 100-gf load and a 10 s dwell time. Data were analyzed by one-way ANOVA followed by Student–Newman–Keuls multiple comparison test.

Results: All irrigation solutions except Saline produced a significant decrease in microhardness ($p<0.05$). The greatest percentage of hardness loss was observed in the Propolis group (24.24%), followed by DR HEDP (20.61%) and EDTA (19.56%). The Saline group showed no significant change (0.81%). No statistically significant differences were found among the EDTA, DR HEDP, and Propolis groups ($p>0.05$).

Conclusion: Although not statistically significant, Dual Rinse HEDP caused a greater decrease in root dentin microhardness than EDTA.

Keywords: Dentin microhardness; dual rinse HEDP; propolis; root canal irrigation; Vickers hardness test.

Introduction

The fundamental aim of root canal treatment is to eliminate microorganisms from the root canal system and to achieve a hermetic seal by combining mechanical instrumentation with chemical disinfection, a process known as chemomechanical preparation (1,2). Due to the complex morphology of the root canal system and the inaccessibility of certain areas to mechanical instrumentation, irrigation protocols play a crucial role in the success of endodontic treatment (3). During mechanical instrumentation, a smear layer composed of organic and inorganic components—including dentin debris, pulp tissue remnants, and bacteria—is formed on the root canal walls. This smear layer may hinder the penetration of irrigation solutions into the dentinal tubules, thereby compromising disinfection and the bonding ability of root canal sealers to the canal walls (4-6).

Sodium hypochlorite (NaOCl) is the most widely preferred root canal irrigating solution due to its strong antimicrobial activity and ability to dissolve organic tissues. However, its inability to effectively remove inorganic components necessitates the use of a chelating agent (7). A commonly adopted approach is final irrigation with ethyl-

Cite this article as: Ozasir B, Ozasir T, Yesil I, Arslan ZS, Gulsahi K. Effect of dual rinse® HEDP, propolis and EDTA on root dentin microhardness: An in vitro study. Turk Endod J 2025;10:218-223.

Correspondence: Birgül Özaşır. Department of Endodontics, Baskent University Faculty of Dentistry, Ankara, Türkiye
Tel: +90 312 – 246 66 15 e-mail: beren@baskent.edu.tr

Submitted: June 20, 2025 Revised: July 07, 2025 Accepted: July 11, 2025 Published: December 09, 2025

enediaminetetraacetic acid (EDTA). Nevertheless, EDTA has some disadvantages, including incomplete smear layer removal in the apical region, the requirement for sequential use with NaOCl due to chemical interactions that reduce the efficacy of available chlorine, and the potential for calcium ion loss and reduced dentin microhardness with prolonged use (8).

Certain irrigation solutions have been reported to alter the chemical structure of dentin, especially affecting the calcium/phosphorus (Ca:P) ratio (9). These alterations may negatively impact dentin permeability and solubility, ultimately reducing the adhesion quality of root canal filling materials. This could, in turn, lead to decreased sealer penetration and an increased risk of apical microleakage (10). To overcome the limitations associated with conventional chelators like EDTA, a "soft chelating agent" known as etidronic acid (1-hydroxyethane-1,1-diphosphonic acid; HEDP) has been proposed as an alternative (8). Dual Rinse® (DR HEDP; Medcem, Weinfelden, Switzerland), a commercially available powder formulation containing 9% HEDP, has been shown to maintain active chlorine levels for up to one hour when mixed with NaOCl (11). This formulation supports continuous chelation, preventing smear layer formation in areas where instruments contact the canal walls and debris accumulation in non-contact areas, thereby eliminating the need for sequential irrigation protocols (12). One of the irrigants evaluated in this study was propolis, a resinous substance naturally produced by bees. Rich in flavonoids, propolis contains a complex mixture of phenolic compounds, esters, terpenoids, beta-sterols, aromatic aldehydes, and alcohols. Its broad-spectrum biological properties, including antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, hepatoprotective, and immunomodulatory effects have been studied. Notably, propolis has shown promising efficacy against endodontic pathogens, even under challenging conditions such as the presence of smear layer, indicating its potential as an alternative to traditional irrigation solutions such as NaOCl. (13). Studies comparing propolis to NaOCl have shown that propolis possesses similar antibacterial activity and remains effective even in the presence of smear layer (14).

To our knowledge, no previous study has compared the effects of propolis and DR HEDP on root dentin microhardness. Therefore, this study aimed to evaluate and compare the effects of DR HEDP, propolis, and EDTA on root canal dentin microhardness as it may influence clinicians' irrigation preferences in practice. The null hypothesis was that the irrigation solutions would not significantly affect dentin microhardness.

Materials and Methods

This study followed the updated principles of the Declaration of Helsinki and was approved by the Non-Interventional Clinical Research Ethics Committee of Başkent University (Date: 25/06/2025, No: 25/153). It was also approved by the Medical and Health Sciences Research Board of Başkent University (Project No: D-DA25/08) and supported by the Başkent Research Fund.

Sample size determination

Power analysis was performed using G*Power 3.1 (Heinrich Heine University, Düsseldorf, Germany) based on the microhardness data reported by Dineshkumar et al. (15). A large effect size (f=0.40) was assumed, and it was determined that at least 19 samples per group were needed at a 0.05 error level and 80% power.

Tooth selection and sectioning

Forty extracted single-rooted human maxillary central incisors, extracted for orthodontic or periodontal reasons, were selected. Soft tissue remnants were cleaned using periodontal curettes, and the teeth were stored in distilled water until use. Digital radiographs were taken to verify the absence of calcifications and complex canal anatomy and to examine root curvature. Teeth with root curvature greater than 10° were excluded from the study. Crowns were removed at the cementoenamel junction under water cooling using a diamond disc, and roots were then split buccolingually. Each section was embedded horizontally in acrylic resin molds with the dentin surface exposed.

Preparation and Polishing

The exposed dentin surfaces were ground using a Presi Mecapol P230 grinder (Presi, Grenoble, France) with silicon carbide abrasive papers of P600, P800, and P1000, respectively, (Metkon Instruments Ltd., Bursa, Türkiye) under water cooling. Final polishing was done using felt discs and polishing paste (Preparations Diamantees Mecaprex, KMV, Grenoble, France).

Experimental Groups and Irrigation Protocols

In this study, saline, EDTA, DR HEDP, and propolis irrigants were used for microhardness measurement. The samples were randomly assigned to groups using a computer-generated randomization list created in Microsoft Excel. Each sample was given a unique number, and the RAND() function was used to assign them randomly to the experimental groups as follows:

Group 1: Saline (0.9% saline for 20 min - distilled water for 1 min)

Group 2: EDTA (20 min 2.5% NaOCl - 1 min distilled water- 1 min 17% EDTA - 1 min distilled water)

Group 3: DR HEDP (20 min DR HEDP - 1 min distilled water)

Group 4: Propolis (20 min 2.5% NaOCl - 1 min distilled water- 4 min 4% propolis - 1 min distilled water)

Preparation of irrigation solutions

Saline solution (Polifleks®, Polifarma Pharmaceutical Co., Türkiye) was used for Group 1 (control). For Groups 2 and 4, 2.5% NaOCl was prepared by mixing distilled water and 5% NaOCl (Microvem, Sakarya, Türkiye) at a 1:1 ratio. For Group 2, 17% ethylenediaminetetraacetic acid solution (AQUA-EDTA 17%, AQUA Medikal, Ankara, Türkiye) was used. For Group 3, 9% Dual Rinse® HEDP (Medcem, Weinfelden, Switzerland) solution was obtained by mixing 1 capsule (0.9 g) of etidronic acid powder with 10 mL of 2.5% NaOCl with a plastic spatula for 2 min, in accordance with the manufacturer's recommendations. For Group 4, 4% propolis solution was obtained by dissolving 500 mg tablet (Bee'O Up®, Bee&You Propolis Tablet, BEE'O, Istanbul, Türkiye) in 120 mL of warm distilled water. All solutions were prepared fresh and stored in the dark before the experiments. Each solution was applied in a volume of 5 mL to the dentin surfaces, and each group was rinsed with distilled water for 1 min between different solutions if the protocol requires.

Microhardness Test

All microhardness tests were conducted by a single operator blinded to the irrigation groups, while a second blinded operator recorded the pre- and post-irrigation microhardness measurements. Microhardness testing was conducted using a Vickers microhardness tester (HMV-700, Shimadzu Corp., Tokyo, Japan). A pyramidal tip was applied to the surface of each section for 10 seconds with a 100 gram-force (gf) load. The diagonals of the pyramidal scar formed on the section surface were measured under a stereomicroscope at x40 magnification. Representative microscopic images of the traces created by Vickers indentations in different regions of the root canal dentin are presented in Fig. 1. The diagonal lengths of these indentations were measured to calculate the Vickers hardness value (HV). For each sample, the average HV value obtained from three regions (coronal, middle, apical) was used. Pre-irrigation values were recorded, followed by irrigation procedures and post-irrigation measurements.

Statistical Analysis

Descriptive statistics were calculated as mean ± standard deviation, along with minimum and maximum values for each group. Kruskal–Wallis test was used to analyze the initial microhardness values, since they did not meet the assumption of normality. The Shapiro–Wilk test confirmed that both absolute and percentage change values were normally distributed ($p>0.05$), so these variables were analyzed using One-Way ANOVA, followed by Student–Newman–Keuls post-hoc test to determine pairwise differences. The significance level of $p<0.05$ was considered statistically significant. All analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA).

Results

The effects of four different irrigation groups on dentin microhardness were evaluated using the Vickers microhardness test before and after the irrigation protocols. There was no statistical difference between initial microhardness values ($p>0.05$). Both the absolute and percentage changes were calculated for each group along with mean and standard deviation values. In terms of absolute change in microhardness compared to the initial values, a decrease of 1.62±5.83 was detected in the saline group, 11.01±5.57 in the EDTA group, 13.27±8.01 in the DR HEDP group and 13.38±5.83 in the Propolis group. When the percentage change was examined, a decrease of 0.81±15.30% was observed in the Saline group, 19.56±8.63% in the EDTA group, 20.61±10.99% in the DR HEDP group and 24.24±8.32% in the Propolis group, respectively. Table 1 shows the average microhardness values and the change

Table 1. Descriptive statistics of microhardness values before and after irrigation

	N	Initial HV Mean ± SD (Min-Max)	Final HV Mean ± SD (Min-Max)	ΔHV Mean ± SD	%ΔHV Mean ± SD
Saline	20	49.58±9.78 (25.80–60.80)	47.96±6.26 (36.40–55.60)	1.62±5.83*	0.81±15.30*
EDTA	20	55.51±8.11 (45.20–75.40)	44.50±7.19 (34.30–63.30)	11.01±5.57	19.56±8.63
DR HEDP	20	59.60±12.35 (37.50–82.30)	46.33±6.20 (37.80–62.70)	13.27±8.01	20.61±10.99
Propolis	20	53.77±8.00 (30.20–68.40)	40.39±5.53 (26.30–48.70)	13.38±5.83	24.24±8.32

N: number of samples; HV: Vickers Hardness Value; SD: standard deviation; Min: minimum; Max: maximum; ΔHV: Absolute change in microhardness; %ΔHV: Percentage change in microhardness. *Statistically significant difference ($p<0.05$, Student–Newman–Keuls test).

rates in microhardness before and after the irrigation protocols for each solution group. The decrease in microhardness compared to the initial value was significant in the groups except the Saline group ($p<0.001$). However, no statistically significant difference was found between the EDTA, DR HEDP and Propolis groups ($p>0.05$).

Discussion

In this study, the effects of 17% EDTA, which is frequently used in root canal irrigation, as well as DR HEDP and propolis, a natural product, on root dentin microhardness were evaluated. Present findings showed that all experimental groups, except the control (Saline), significantly reduced dentin microhardness, partially rejecting the null hypothesis.

The highest reduction was observed in the Propolis group, followed by DR HEDP and EDTA with no statistically significant differences. Dentin microhardness is clinically important for both the mechanical integrity of root canal walls and the bonding strength of root canal sealers. A decrease in microhardness may increase the brittleness of dentin, reducing the tooth's resistance to occlusal forces and compromising the prognosis. It may also reduce the adhesion of resin-based sealers (16,17). The Vickers microhardness test was chosen due to its precision in measuring small samples and sensitivity to physical changes caused by chemical agents (16). Consistent with previous studies (18,19), both pre- and post-irrigation microhardness values were recorded and compared with the control group.

To simulate clinical practice, all specimens were exposed to NaOCl. In the continuous chelation group, HEDP was mixed with NaOCl, whereas EDTA and propolis were applied after NaOCl in the sequential groups. All specimens were exposed to NaOCl for 20 min, approximating the duration of root canal instrumentation and tissue dissolution in clinical procedures. DR HEDP and EDTA produced similar reductions in microhardness. Literature findings vary, with some studies (2,20-23) reporting that DR HEDP causes less mineral loss than EDTA, while Grinkevičiūtė et al (24) observed greater microhardness loss with DR HEDP. The discrepancies may be due to differences in application time and methodology (22-25).

According to the present results DR HEDP caused a comparable decrease to EDTA, although used for a much longer time (20 min vs. 1 min). While HEDP is a weaker chelator compared to EDTA (2), its prolonged exposure may have compensated for this by gradually removing mineral content over time. The contact of chelating agents with dentin may lead to mineral loss through calcium and phosphate ion release, which can alter the structural and chemical integrity of dentin (20). In the present study, such chemical interactions may have contributed to the observed reduction in microhardness.

The different results of the effects of EDTA and DR HEDP groups on dentin microhardness between the present work and the study of Hazar et al. (2) may be due to the differences in the method used, irrigation time and measurement strategy. In this study, the average HV value was calculated by combining the microhardness measurements taken from the coronal, middle and apical regions of each sample and the analysis was performed on this single average value. In contrast, the study by Hazar et al. (2) evaluated each of the three root regions separately, and the region-specific effects were statistically analyzed. Furthermore, the application of EDTA was different in the present work (1 min instead of 2). These factors may underlie the differences in the findings.

This study's results showed that the Propolis group caused the highest decrease in dentin microhardness, although not at a statistically significant level. This result is consistent with the results of Elgendy et. Al (13). Unlike the present work, 4% propolis solution dissolved in dimethyl sulfoxide (DMSO) instead of distilled water caused a greater decrease in dentin microhardness than 17% EDTA. However, in the study by Bhagwat et al. (25), 4% propolis solution prepared with distilled water was found to be less effective on dentin microhardness than 17% EDTA. These contradictory findings may be related to formulation differences resulting from the natural structure of propolis. Ahangari et al. (14) reported that the composition of propolis varies significantly depending on factors such as source, extraction method (water-based or ethanol-based) and pH profile, which may directly affect the biological effects of propolis. In this context, it is difficult to make a direct generalization among the studies in the literature evaluating the effects of propolis on dentin microhardness. Therefore, the significant reduction observed in this study may have been shaped by the formulation, application time and solubility properties of propolis.

No study has been found in literature directly comparing HEDP and propolis in terms of dentin microhardness. Therefore, this study is the first to compare the structural effects of these two irrigants on dentin. This study demonstrates that EDTA, HEDP, and propolis may have similar negative effects on structural integrity when assessed in terms of dentin microhardness. Although HEDP and propolis formulations have been reported to be more protective on dentin microhardness in some studies (20-23), factors such as application method, duration and concentration can significantly affect the results (24).

This in vitro study has several limitations that should be

acknowledged. Firstly, experimental conditions cannot replicate the complex biological environment of the oral cavity, particularly the influence of pulp tissue, physiological fluids, and intraoral temperature fluctuations. Secondly, although microhardness was assessed in three distinct regions (coronal, middle, and apical), the data were averaged for statistical analysis, potentially masking region-specific effects of the irrigation solutions. Moreover, the irrigation protocols and exposure times were standardized across groups for comparison but may not accurately reflect clinical practice where variability in application duration and technique exists. The chemical composition and concentration of propolis may also differ depending on its botanical source and extraction method, limiting the generalizability of the findings. Lastly, the sample size, though statistically powered, remains relatively small and may not capture the full biological variability of human dentin. Further in vitro and vivo studies that simulate clinical conditions are recommended to confirm these results and to explore strategies for minimizing adverse effects on dentin while maintaining adequate canal disinfection.

Conclusion

All tested irrigating solutions in this study reduced root dentin microhardness compared to the saline control, indicating that each can weaken the dentin structure. Among the experimental groups, the natural propolis irrigation solution produced the greatest reduction in dentin hardness, whereas the conventional chelator EDTA showed the mildest reduction; however, these differences were not statistically significant. In other words, continuous chelation with HEDP and the alternative herbal agent propolis were not inherently safer for dentin microhardness than EDTA, as all three agents exerted a similar negative impact on the structural integrity of dentin.

Authorship Contributions: Concept: B.O., T.O., I.Y., Z.S.A., K.G.; Design: B.O., T.O., I.Y., Z.S.A., K.G.; Supervision: B.O., T.O., I.Y., Z.S.A., K.G.; Fundings: T.O. B.O.; Materials: I.Y., Z.S.A.; Data: B.O., T.O., I.Y., Z.S.A., K.G.; Analysis: T.O., K.G.; Literature Search: B.O., T.O., I.Y., Z.S.A., K.G.; Writing: B.O.; Critical Revision: B.O., T.O., I.Y., Z.S.A., K.G.

Use of AI for Writing Assistance: Not declared

Source of Funding: None declared.

Conflict of Interest: None declared.

Ethical Approval: Approved by Conventional Clinical Research Ethics Committee of Başkent University (Date: 25/06/2025, No: 25/153).

Informed consent: Written informed consent was obtained from patients who participated in this study.

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