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ORIGINAL ARTICALE

IMPACT OF QUERCETIN AS AN ENDODONTIC IRRIGANT ON THE MICROHARDNESS AND FLEXURAL STRENGTH OF RADICULAR DENTIN – AN IN VITRO STUDY

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ABSTRACT

Background: Successful root canal therapy relies on effective irrigation protocols that disinfect while preserving dentin integrity. Conventional irrigants such as sodium hypochlorite (NaOCl) and Ethylene-Diamine-Tetraacetic-Acid (EDTA) are effective but compromise dentin microhardness and mechanical properties. Quercetin, a plant-based natural flavonoid, has antimicrobial and collagen-stabilizing effects, making it a potential biocompatible alternative for Synthetic ones. Hence, the present in-vitro study was carried out to evaluate the effect of quercetin as an endodontic irrigant on the microhardness and flexural strength of radicular dentin in comparison with NaOCl and EDTA.

Materials and Methods: Forty eight extracted human anterior teeth were prepared into dentin specimens and randomly assigned to four groups: Group A (saline), Group B (3% NaOCl with 17% EDTA), Group C (5% NaOCl with 17% EDTA), and Group D (6.5% quercetin). Microhardness was measured using a Vickers hardness tester (VHN- Vickers Hardness Number), and flexural strength was evaluated using a three-point bending test (MPa). Data were analyzed using ANOVA, paired t-tests, and Tukey's Honestly Significant Difference (HSD) test at significance set at $p < 0.05$.

Results: Quercetin irrigation (Group D) significantly increased dentin microhardness (61.09 ± 3.38 VHN) and flexural strength (95.23 ± 1.53 MPa) compared to other groups ($p < 0.001$). NaOCl with EDTA, particularly at 5%, caused the greatest reduction in both parameters.

Conclusion: Quercetin showed superior preservation and reinforcement of dentin mechanical properties compared to conventional irrigants, highlighting its potential as a safe and effective endodontic irrigant.

Keywords: Endodontic irrigant, Flexural strength, Microhardness, Plant-based Irrigant, Quercetin.

INTRODUCTION

Effective irrigation during root canal therapy is a crucial step that determines its success. Conventional irrigants such as sodium hypochlorite (NaOCl), Ethylene-Diamine-Tetraacetic-Acid (EDTA), and chlorhexidine (CHX) have well-documented antimicrobial and chelating properties, yet their associated adverse effects on dentin, microhardness, collagen degradation and compromised fracture resistance have cautioned their usage [1–3]. Thus, to combat this, the natural biocompatible alternatives that disinfect without weakening dentin have been explored over the years.

Quercetin is a flavonoid found abundantly in fruits and vegetables, that possess antioxidant, anti-inflammatory and microbial properties [4–6]. It was found to have potent anti-bacterial action against *Enterococcus faecalis*, a major cause of endodontic failure [7, 8]. It also acts as a collagen cross linker and stabilizes the Dentin's organic matrix [9]. Laboratory studies have shown that quercetin based irrigants improved dentin microhardness in dose dependent manner and mitigated the deleterious effects of NaOCl and outperformed CHX [2, 10, 11]. Studies have also shown the collagen stabilizing effect of Quercetin preserved the dentin's flexural strength and improved fracture resistance [8, 12].

Despite the growing interest in natural and biocompatible alternatives to conventional chemical irrigants, there remains a significant scarcity of research specifically evaluating the effects of quercetin on the structural and mechanical properties of radicular dentin. While agents like sodium hypochlorite and EDTA are widely studied, their adverse impact on dentin integrity raises the need for safer alternatives. Quercetin, with its reported antioxidant, antimicrobial, and collagen-stabilizing properties, presents a promising candidate; however, limited evidence exists regarding its influence on dentin microhardness and flexural strength, the two critical factors for long-term endodontic success. In light of this gap, the present in-vitro study was undertaken to comprehensively assess Quercetin's potential as an effective, biologically safe, and mechanically favorable endodontic irrigant.

MATERIALS AND METHODS

1. Sample Collection and Preparation:

A total of 48 extracted human anterior teeth were collected from various dental clinics across the city following aseptic protocol and collection guidelines [13]. Sound teeth, free from caries, restorations, fractures or developmental anomalies were included for the study. Adherent soft tissue remnants were carefully removed using Waldent Max Piezo 2 Ultrasonic Scaler (Waldent Innovations Pvt. Ltd., India) and then the teeth were thoroughly rinsed with distilled water.

2. Crown Sectioning and Sample Allocation:

The teeth were sectioned at the cementoenamel junction using a low speed diamond disc under water to prevent thermal damage. The specimens were then randomly allocated into two groups. For Microhardness testing 32 specimens were included and for flexural strength testing 16 specimens were included.

3. Preparation for Microhardness Evaluation:

For microhardness testing the roots of 32 anterior teeth were transversely sectioned using low-speed precision disc cutter under water cooling and a discs of 4mm thickness were prepared. The disc surface was sequentially grounded using silicon carbide abrasive papers of increasing grit sizes (600, 800, and 1200 respectively). Polishing of the grounded surface using alumina slurry was done to achieve a standardized smooth surface suitable for indentation. Caution was taken to minimize surface irregularities and heat generation during procedure.

4. Preparation for Flexural Strength Evaluation:

For flexural strength testing,16 anterior teeth were sectioned longitudinally using a diamond saw under water irrigation into 32 halves and a specimen of 12 mm × 2 mm × 2 mm (length × width × thickness) was prepared using a precision cutting machine and standardized using the digital caliper. The surfaces were polished to eliminate irregularities and surface defects. All prepared specimens were stored in distilled water at room temperature for 24 hours before further experimental procedures.

5. Grouping and Experimental Design: The specimens were randomly assigned to four experimental groups for Microhardness (n=8) and Flexural strength (n=8) testing. Group A received Saline (control) irrigant, Group B was treated with 3% Sodium hypochlorite (NaOCl) with 17% EDTA, Group C used 5% Sodium hypochlorite (NaOCl) with 17% EDTA and Group D had 6.5% Quercetin solution as irrigant.

6. Irrigant Preparation: The 6.5% quercetin solution was prepared by dissolving 6.5 g of pure quercetin powder (Sigma–Aldrich, St. Louis, MO, USA) in 100 mL of absolute ethanol. The solution was then placed in water bath at 37 °C for 15 minutes to ensure complete dissolution of the powder before use.

7. Irrigation Procedure: Irrigation of the specimen was done using the 2ml syringe fitted with 30 gauge side-vented needle. Each specimen was irrigated for 5minutes of the assigned irrigant followed by distilled water rinse for 1 minute and gently dried with filter paper to remove residual moisture.

8. Mechanical Testing: For Flexural strength testing, a standardized bar shaped specimen were subjected to three-point bending using a Universal Testing Machine (Instron, USA) at a crosshead speed of 1 mm/min. The maximum load at fracture was recorded in Newtons (N), and flexural strength was calculated in megapascals (MPa). To test microhardness, dentin discs were subjected to indentation using a Vickers microhardness tester (Vickers （Micro） Hardness-Tester- HV-5103050A, load: 100 g; dwell time: 15 seconds) by Laizhou Jincheng Industrial Equipment Co., Ltd. Three indentations were made at standardized distances from each other, and the mean value was considered as the Vickers Hardness Number (VHN) for each sample.

9.Statistical Analysis: The recorded values for microhardness and flexural strength were tabulated and analyzed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA). The data were described using mean and standard deviation and inferred using t test, ANOVA followed by Tukey's Honestly Significant Difference test (Post-hoc Tukey's HSD test). A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 48 anterior teeth specimens were analyzed for microhardness and flexural strength following irrigation with different test solutions groups (A, B, C, and D)

1. Microhardness (VHN) Analysis:

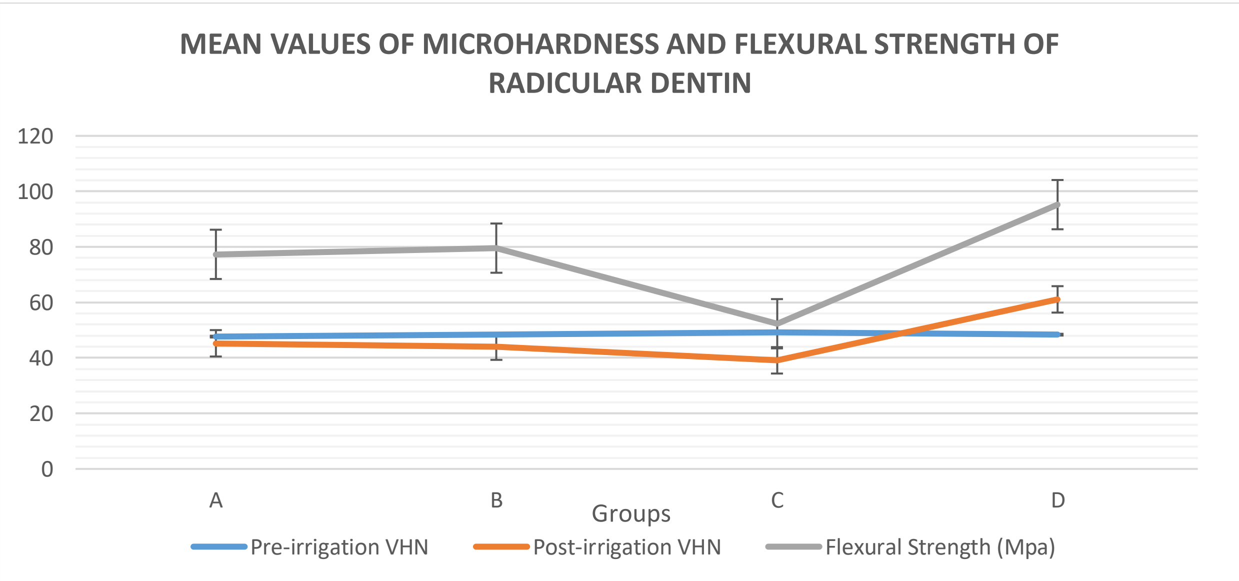
Post-hoc Tukey's HSD analysis showed that Group D (6.5% quercetin) had the highest post-irrigation VHN (61.09 ± 3.38) (Graph 1), which was significantly greater than all other groups ($p < 0.001$) (Tables 1 and 2). At baseline, there were no significant differences in VHN values between the groups ($p = 0.936$). After irrigation, however, clear differences emerged ($p < 0.001$), with Group C (5% NaOCl + 17% EDTA) showing the greatest reduction in microhardness (39.13 ± 3.76) (Table 1). Paired t-test results confirmed that Groups A, B, and C experienced significant decreases in VHN ($p < 0.05$), while Group D showed a significant increase ($p < 0.001$) (Tables 3 and 4) suggesting that quercetin not only preserves but can also enhance dentin microhardness, in contrast to conventional irrigants that compromise dentin integrity.

2. Flexural Strength Analysis:

One-way ANOVA revealed a highly significant difference in flexural strength values among the groups ($p < 0.001$). Group D (6.5% quercetin) showed the highest flexural strength (95.23 ± 1.53 MPa), which was significantly greater than that of Groups A (77.32 ± 12.66 MPa), B (79.56 ± 2.90 MPa), and C (52.31 ± 1.35 MPa) (Table 1, Graph 1). Group C (5% NaOCl + 17% EDTA) demonstrated the lowest flexural strength, which was significantly lower than all other groups ($p < 0.001$) (Table 2). No significant difference was noted between Groups A and B ($p = 0.903$) (Table 5) indicating that quercetin not only prevents loss of dentin strength but also enhances its flexural resistance, whereas conventional irrigants, particularly the NaOCl-EDTA combination, substantially weaken dentin.

Table 1. Descriptive Analysis of microhardness and flexural strength of radicular dentin

Group	Pre-irrigation VHN (Mean ± SD)	Post-irrigation VHN (Mean ± SD)	Flexural Strength (MPa, Mean ± SD)
A	47.68 ± 6.42	45.26 ± 6.48	77.32 ± 12.66
B	48.34 ± 4.17	44.05 ± 4.70	79.56 ± 2.90
C	49.21 ± 3.80	39.13 ± 3.76	52.31 ± 1.35
D	48.38 ± 4.28	61.09 ± 3.38	95.23 ± 1.53



Graph 1. Mean values of microhardness and flexural strength of radicular dentin

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Table 2. One-way ANOVA results for microhardness and flexural strength of radicular dentin

Parameter	Source of Variation	Sum of Squares	df	Mean Square	F	p-value
Pre-irrigation (VHN)	Between groups	9.523	3	3.174	0.139	0.936
Post-irrigation (VHN)	Between groups	2172.906	3	724.302	32.348	<0.001*
Flexural strength (MPa)	Between groups	7561.049	3	2520.350	58.328	<0.001*
***p<.05- Statistically Significant**						

Table 3. Paired sample t-test results for pre- and post-irrigation VHN of radicular dentin

Group	Mean Difference ± SD (Pre – Post)	t	df	p-value
A	2.41 ± 0.82	8.34	7	<0.001*
B	4.29 ± 3.39	3.57	7	0.009*
C	10.09 ± 4.07	7.01	7	<0.001*
D	–12.71 ± 3.44	–10.46	7	<0.001*
***p<.05- Statistically Significant**				

Table 4. Post-hoc Tukey's HSD Test for multiple comparisons of Post-irrigation microhardness (VHN) of radicular dentin:

Comparison	Mean Difference	Std. Error	p-value	95% CI (Lower– Upper)
A – B	1.21	2.37	0.955	–5.25 to 7.67
A – C	6.14	2.37	0.067	–0.32 to 12.60
A – D	–15.83	2.37	<0.001*	–22.29 to –9.37
B – C	4.93	2.37	0.184	–1.54 to 11.39
B – D	–17.04	2.37	<0.001*	–23.50 to –10.58
C – D	–21.96	2.37	<0.001*	–28.42 to –15.50
***p<.05- Statistically Significant**				

Table 5. Post-hoc Tukey's HSD Test for multiple comparisons of Flexural Strength of radicular dentin:

Comparison	Mean Difference	Std. Error	p- value	95% CI (Lower–Upper)
A – B	–2.24	3.29	0.903	–11.22 to 6.73
A – C	25.01	3.29	<0.001*	16.03 to 33.98
A – D	–17.91	3.29	<0.001*	–26.88 to –8.93
B – C	27.25	3.29	<0.001*	18.27 to 36.22
B – D	–15.67	3.29	<0.001*	–24.64 to –6.69
C – D	–42.91	3.29	<0.001*	–51.89 to –33.94
***p<.05- Statistically Significant**				

DISCUSSION

The present study evaluated the influence of Plant-based and synthetic endodontic irrigants on dentin microhardness and flexural strength. The study findings revealed that Group D (6.5% quercetin) demonstrated significantly increased dentin microhardness and flexural strength compared to sodium hypochlorite and EDTA-based irrigants. A significant increase in dentin microhardness (61.08 ± 3.37 VHN) and flexural strength (95.22 ± 1.53 MPa) following irrigation with 6.5% quercetin observed in the present study was consistent with a previous observations by Abdou and Azab where 6.5% quercetin irrigation significantly increased the microhardness of 64.83± 0.95 compared to the other groups with 2% quercetin (62.78± 0.7) and 2% CHX (53.36±1.1) respectively[2]. This is attributed to the inherent potential of Quercetin to not only preserve the dentinal structure but also its ability to cross link dentin collagen. Liu et al in their study similarly found that quercetin can bind to collagen fibres, leading to the formation of cross-linkages that stabilize the organic matrix and protect it from degradation by matrix metalloproteinases (MMPs) and enhances the mechanical properties of the dentin [8].

In contrast to these beneficial effects of quercetin, Group C was associated with most significant decrease in microhardness (39.12 ± 3.75 VHN) and the lowest flexural strength (52.31 ± 1.34 MPa). Study done by Marending et al found that sequential irrigation with NaOCl followed by EDTA caused significant reduction in the flexural strength (145.6 ± 30.1) compared to other groups [14]. Haralur SB et al found that irrigation with 5.25% NaOCl and 17% EDTA significantly compromised the microhardness [15]. A study by Mai et al observed that 60 min exposure of NaOCl significantly decreased the flexural strength [16]. A Comprehensive systematic review by Aqabat et al has mentioned that NaOCl interacts with dentin collagen matrix leading to deproteinization in turn leading to reduced dentin hardness and elasticity [17]. Correspondingly in another review by Kumaresan K et al also highlighted that continuous chelation with the NaOCl-HEBP mixture had minimal, less deleterious effect on the physicochemical properties of dentin compared to sequential irrigation with NaOCl followed by EDTA, which may contribute to the long-term survival of the root canal-treated tooth [18].

The observation that Groups A and B exhibited comparable flexural strength values, with no significant difference between them ($p = 0.903$), highlights that conventional or non–collagen-stabilizing irrigants exert a similarly detrimental effect on dentin's mechanical properties. These findings reinforce the critical importance of selecting irrigating solutions that not only provide effective antimicrobial action and smear layer removal but also safeguard the structural integrity of the tooth[19]. Beyond its antimicrobial and collagen-stabilizing effects, quercetin is a plant-derived flavonoid known for its potent antioxidant and anti-inflammatory properties, which may contribute to reducing oxidative stress and protecting the dentin matrix during irrigation. Notably, 6.5% quercetin demonstrated the ability to preserve and even enhance dentin hardness and strength, as evidenced by its highest mean values for both VHN and flexural strength. This dual advantage of mechanical reinforcement and biological safety positions quercetin as a promising alternative to traditional irrigants, particularly for improving the long-term fracture resistance and clinical prognosis of endodontically treated teeth[19,20]. Furthermore, the natural origin and biocompatibility of quercetin offer an added advantage over synthetic agents, aligning with the current trend toward safer, eco-friendly, and patient-friendly endodontic practices.

CONCLUSION

Within the limitations of this study, particularly the inability to fully replicate the dynamic oral environment and its influence on tooth structure following irrigation, it can be concluded that, unlike sodium hypochlorite and EDTA, which are known to adversely affect dentin's structural properties, quercetin demonstrates strong potential as a natural irrigant with antimicrobial activity. Its collagen-stabilizing effect may further enhance dentin integrity, a critical factor for long-term sustainability and durability, thereby offering a distinct advantage over synthetic counterparts.

Moreover, quercetin's biocompatibility and plant-derived origin make it an appealing option for patients seeking safer, naturally based therapeutic alternatives. These findings suggest that quercetin could serve as a promising plant-based substitute for conventional irrigants, contributing not only to improved dentinal reinforcement but also to minimizing potential adverse effects associated with synthetic agents. However, its long-term clinical reliability and safety are yet to be established. Therefore, comprehensive clinical trials incorporating standardized irrigation protocols, larger sample sizes, and extended follow-up periods are essential. Future investigations should also explore optimal delivery methods, effective concentration ranges, and potential synergistic combinations with other irrigants to maximize clinical efficacy and outcomes.

DECLARATION

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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